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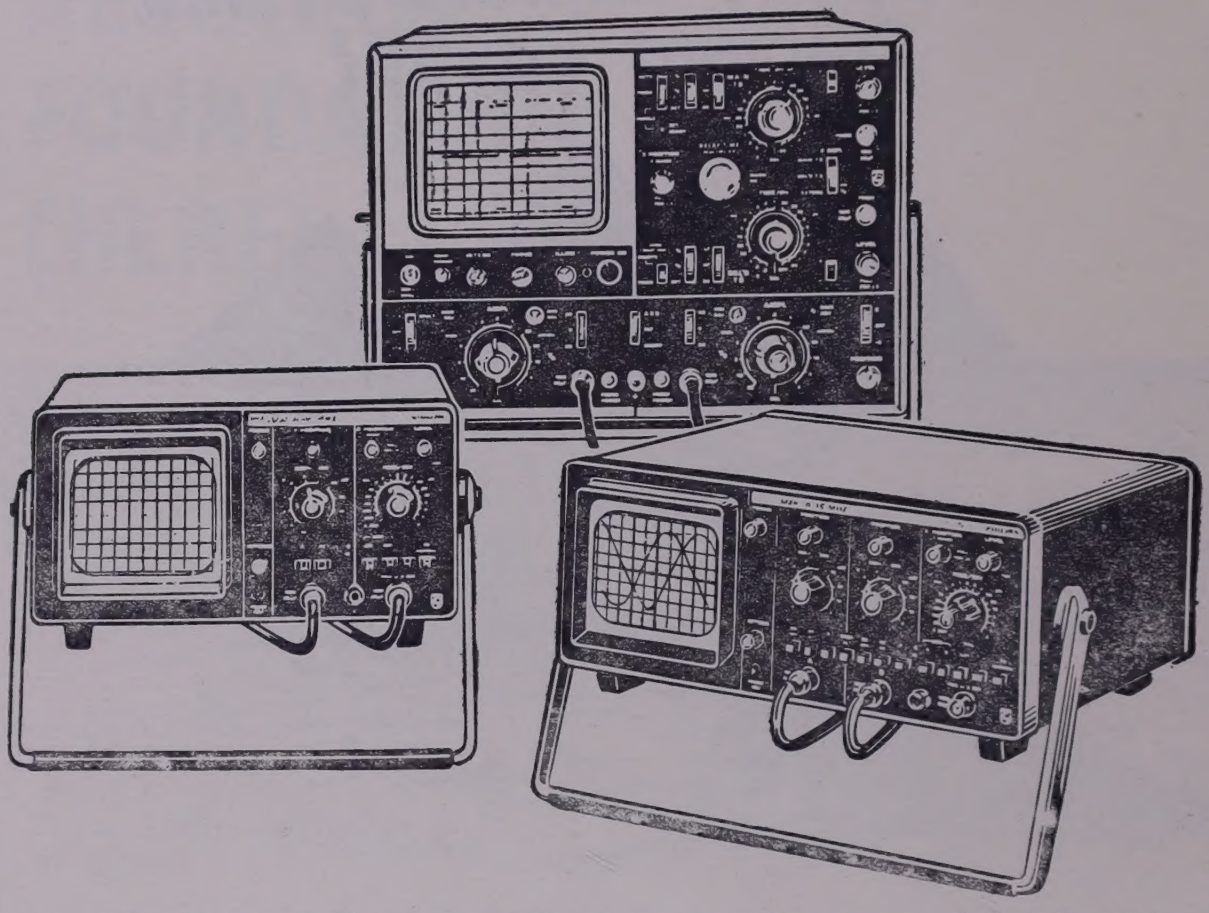

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Foundry Industry of India towards the Year 2000: Technological Forecasting

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Technological forecasting (TF) is a recently developed tool for planning national and corporate production and research and development activities. The technological environment of the world is undergoing rapid changes due to the breakthroughs being achieved in science and technology at ever increasing rates. Therefore, planning activities cannot be carried out efficiently with the assumption that the technologies available for future economic growth will be the same as the ones available today. Thus, accurate anticipation of the future technologies and demands is essential for effective planning. TF basically consists in making a systematic and comprehensive quantitative analysis of the future. The modern TF is a methodology for predicting (i) the technologies that should be developed at different times in the future to meet the specific needs in the light of the likely available resources, and (ii) the technologies that are likely to be available at different times in the future in the light of the present rate of scientific innovation and development.

Quantitative forecasts indicate that R & D inputs and commercialization efforts should be concentrated in certain areas, in preference to other areas. TF is not a substitute for planning, but it could be made into a plan if commitments are made to allocate resources based on the forecasts.

The main techniques used for TF are as follows[1-10].

(i) *Intuitive forecasting* — This includes two methods: (i) Consensus and (ii) Delphi. Both these methods are based on obtaining systematic objective expert opinions. In the former, opinions of individual experts are assessed directly in one interview, while in the latter, a picture of the future is arrived at by soliciting expert opinions through a carefully designed programme of sequential questionnaires. The basis of this technique is that these days discoveries are frequently engineered by sustained inputs of manpower and funds. Therefore, a systematic probing of the minds of experts leads to a time estimate of certain technological happenings. The result of a typical Delphi exercise in the area of iron and steel industry is given in Table 1. The main drawbacks of this method are that it is cumbersome, time-consuming and the value of the results depends upon the selection of the panel members and the extent of their participation.

(ii) *Trend extrapolation* — This involves extrapolation of the existing trends with the assumption that the past trends are likely to continue into the future. The techniques used are simple extrapolation, substitution, semi-logarithmic extrapolation, trend correlation and S-type growth curves. In all these, the pertinent parameters are plotted against time and then extrapolated to reasonable future limits.

The logarithms of technological parameters and production outputs of many technologies in the developed countries have been following linear trends with time. Extrapolations of these linear semi-log plots are more reliable than those of non-linear plots. For example, logarithms of efficiency of illumination, maximum speed of aircrafts, world copper and aluminium productions have varied linearly with time. Similarly, in India, despite the fact that technological growth is likely to be unsystematic due to erratic imports of knowhow, logarithms of numbers of electric motors produced and the value of electronic components produced have increased linearly with time[6]. In the foundry industry, production of spheroidal graphite (SG) iron castings[11] has varied in a logarithmic manner (Fig. 1). These types of linear plots can be extrapolated with some confidence into the future.

TABLE 1 — A TYPICAL DELPHI FORECAST[1]

Sl No.	Potential developments evaluated by percentage of vote distribution for likelihood and impact	Likelihood by 1980						Impact if it occurs by 1980			
		Very probable	Probable	Either way	Improbable	Very improbable	No judgement	Strong	Moderate	Slight or none	No judgement
1	Direct reduction is 10% of steel production										
	Results of Round I	2	24	18	35	0	12	25	19	31	25
	Results of Round II	0	33	34	33	0	0	25	40	20	15
2	Nickel-free stainless steel is 40% or more of stainless steel consumption										
	Results of Round I	17	19	22	11	11	0	6	18	71	6
	Results of Round II	4	38	33	25	0	0	0	32	64	5

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In certain cases, the rate of substitution of one technology by a newer one has varied systematically. Here, the parameter $f/(1-f)$ plotted on semilogarithmic plot varies linearly with time (f is the fraction substituted). For example, Fig. 2 gives the rate at which the open hearth steel making process is being substituted by the electric arc steel making process in USA. In India too, the rate of substitution of steam locomotives by diesel ones has followed a similar trend[6] (Fig. 3). Linearity in $f/(1-f)$ plots will also be obtained in the case of single technology, where one is approaching a limit and the technological parameter plotted is the fraction of the upper limit.

(iii) *Normative approach* — This is a mission oriented approach and here one recedes from the future demands to the present technological imperatives. The future needs and goals are identified and the technologies to meet these demands are deduced. The R & D and other inputs to achieve the necessary breakthroughs and support technologies are specified using decision trees, relevance trees, fine mapping charts and sequence of opportunities and negative (SOON) charts. These charts display the stages, prerequisites, alternatives and road blocks to be overcome before the forecast need can be attained. Such explicit expositions force objectivity and hence the liklihood of meeting the future social demands increases.

(iv) *Technology monitoring* — This is a technique of identifying a useful prospective technology in its embryonic stage and involves extensive analysis of forthcoming innovations, patents and inventions. It also helps in initiating advance action on the future technologies needed by the country. For example[12], a new technology is being developed in Japan for coupling plants producing pig iron by direct reduction of iron ore with nuclear reactor plants. The plants will help in conserving energy as well as coking coal. In 1973, the world steel industry is estimated to have used 11% of the total world consumption of energy[13]. The nuclear reactors could provide hot inert gases, steam and electric power. Hydrogen can be generated using a part of the electric power and used as the reductant for reducing iron ore. This process is highly relevant to India, since our coking coal reserves are of the order of 1·5 billion tonnes, whereas by 1980 coking coal consumption per annum would have risen to about 30 million tonnes per annum. Therefore, monitoring the developments of this type of relevant technologies is important for the Indian foundry industry.

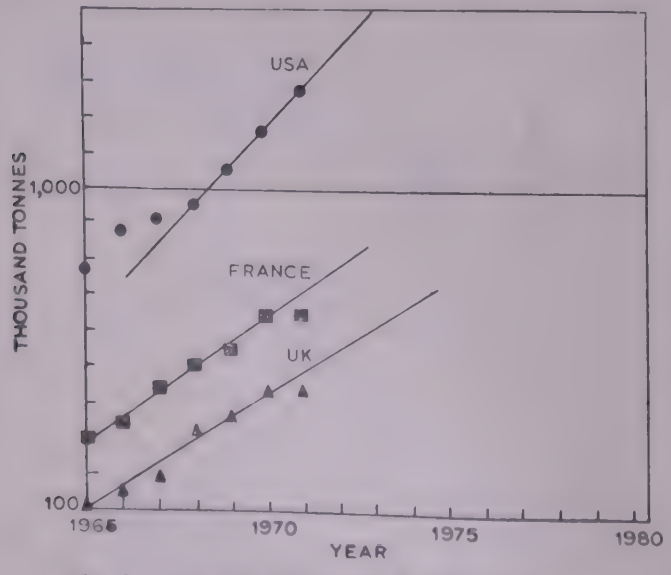

Fig. 1 — SG iron production in some countries

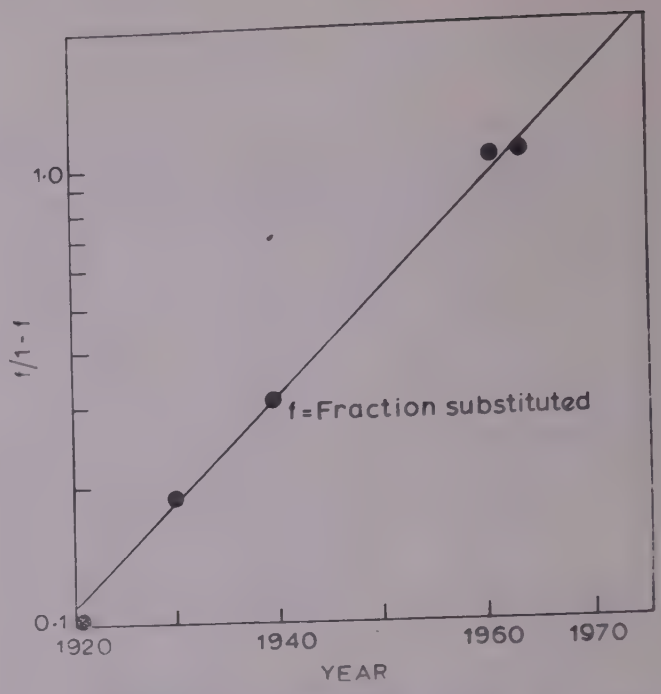

Fig. 2 — Substitution of open hearth process by electric arc process in USA [f, fraction substituted]

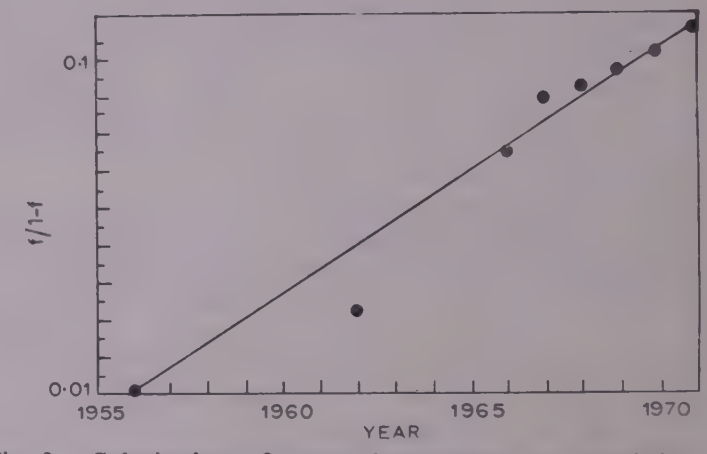

Fig. 3 — Substitution of steam locomotives by diesel locomotives in India

(v) *Dynamic modelling* — In this approach, forecasts are derived on the basis of mathematical models of casual relationships and interactions. It has the drawback of being very expensive and time-consuming in terms of manpower and computer time. However, this is the most logical and quantitative approach to forecasting.

Technology Forecasting as Applied to Indian Foundries

An attempt has been made in this study to forecast the technologies desirable for the foundry industry of India in the year 2000, based on our future needs, present resources and the likely availability of new

technologies. In view of this (i) futuristic assessment of the growth of ferrous foundry industry in India, based on the present production trends and levels of foundry technology is conducted, (ii) problems of the present foundry industry of India are reviewed briefly, (iii) the various foundry forecasts done in other countries are listed and evaluated briefly in the Indian context, (iv) a fine mapping exercise indicating various action imperatives in research and development and in building production infrastructure has been performed, (v) priorities have been assigned to the various imperatives for R & D and production infrastructure using a desirability index score method, and (vi) a possible scenario of Indian foundry industry for the year 2000 has been developed.

Trends in Foundry Industry in India and Forecasts of Production for the Year 2000

Regular foundries existed in India as far back as 1819; however, until 1950, the growth rate was very low. With the launching of the five year plans, the growth rate of foundries has picked up considerably. The demand for castings by the railways had been the main factor in boosting up the foundry industry initially. Till 1966, India had about 4200 iron foundries, 100 non-ferrous foundries, 150 malleable iron foundries and 67 steel foundries[14]. The growth in the number of iron foundries in India was exponential until 1965 (Table 2). However, only about 5000 units of foundries were reported to be existing in 1973[15].

The percentage distribution of the production of various castings during the last few years is shown in Fig. 4. In future, in addition to the total volumes, the percentage distributions will change, specially that of SG iron. The production of major ferrous castings has been derived mainly by the semi-logarithmic trend extrapolation technique using the past data[16–19].

Steel casting — The production of steel castings was growing exponentially from 1952 to 1965, but after that there was a sudden downward shift in the curve (Fig. 5). Exponential growth, similar to that in the period 1952-65, appears to be taking place again. If the present trend continues, the expected production of steel castings is likely to be somewhere between 1700 and 2900 thousand tonnes. Fig. 5 also shows the widening gap between the production of steel castings and their consumption. If the consumption also grows at an exponential rate, as in the past, the import of castings would have to be continued.

It is evident from Fig. 6 that the production of steel castings varied linearly with national income until the year 1965, after which there was a downward shift in the curve. The past trend indicates that the

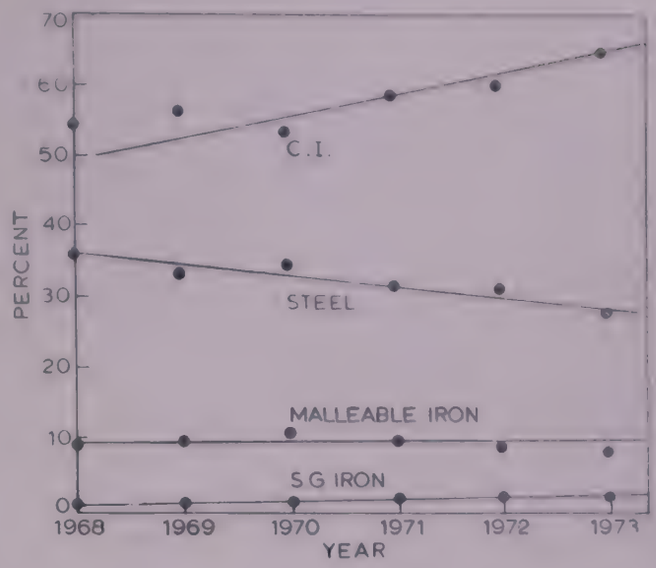

Fig. 4 — Breakup of the production of different types of iron castings in India

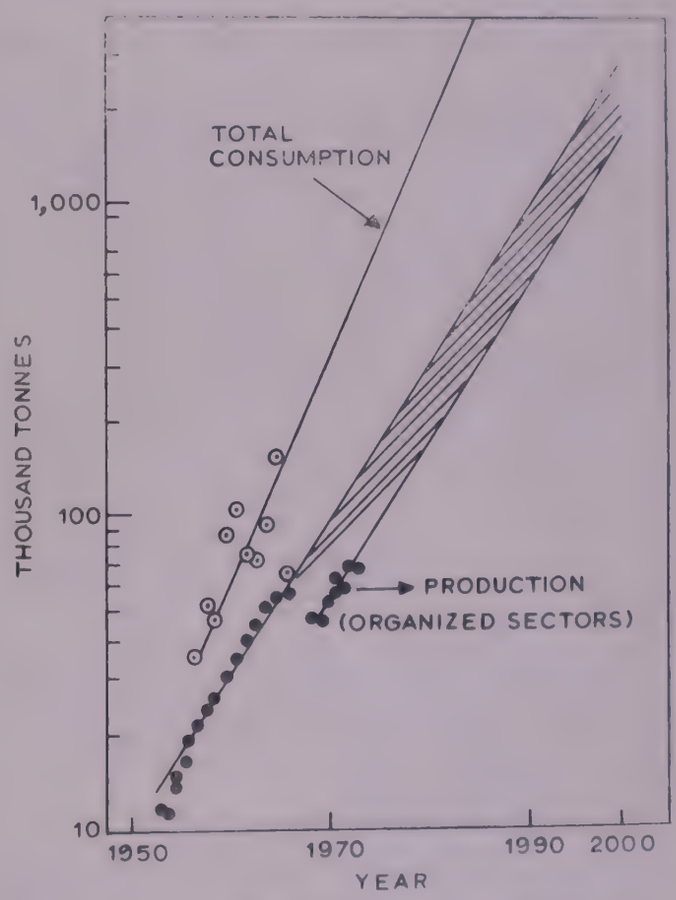

Fig. 5 — Production and consumption of steel castings in India

TABLE 2 — PATTERN OF ESTABLISHMENT OF IRON FOUNDRIES IN INDIA DURING 1819-1965[12]

Period	No. of foundries at the end of the period	Cumulative percentage of total in 1965	Increase %
1819–1850	13	0·3	—
1851–1899	76	1·8	1·5
1900–1925	294	7·0	5·2
1926–1950	1660	39·5	32·5
1951–1955	2245	51·14	12·64
1956–1965	4197	100·00	48·86

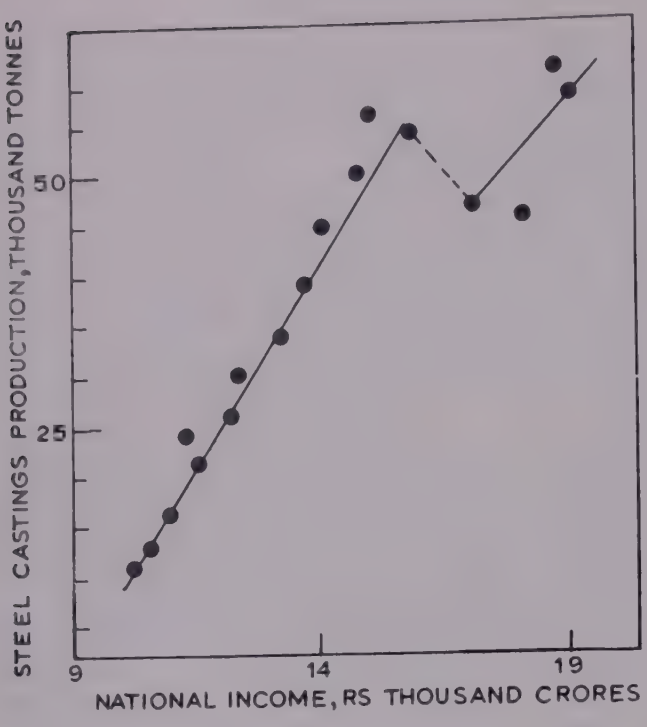

Fig. 6 — Steel castings production vs national income of India

projected values of national income could be used as an indicator for forecasting the production of steel castings. Likewise, other relevant trend correlations can be established and used for extrapolation. For example, growth in production of iron castings can be predicted by plotting the ratio of fraction of value added by foundries producing iron castings to GNP; in fact, any parameter on which the technology is substantially dependent or related can be used. Also, the total demand for steel castings in the future can be arrived at by detailed sectorial demand projections (e.g., demand projections of railways, defence, metallurgical machine industries, etc.) and adding them up for different times in future. However, in this study, these alternative methods of forecasting could not be used due to lack of readily available data. It is preferable to use different methods for cross-checking the different results obtained.

Cast iron — Production of cast iron has been growing exponentially during the period 1968-1973 (Fig. 7). An extrapolation of this trend indicates that the production of cast iron castings is likely to be 700 thousand and 1·8 million tonnes in the years 1980 and 1985 respectively.

Malleable iron — It is seen from Fig. 8 that there has been exponential growth in the production of malleable iron castings; however from the year 1965-66 there has been a sudden downward shift similar to that in the case of steel castings (Figs 5 and 6). However, projecting on the basis of recent trends, 50,000 tonnes of malleable iron castings are likely to be produced by 1980.

SG iron — The production of SG iron castings has been growing exponentially since 1965 (Fig. 9). It would reach about 70,000 tonnes in 1985 and about 25,00,000 tonnes in 1990, based on the semilogarithmic trend. SG iron can substitute both steel and malleable iron in several applications. Therefore, its production has been increasing at an exponential rate all over the world. Figs 10 and 11 show the trends of substitution of steel castings and malleable iron castings

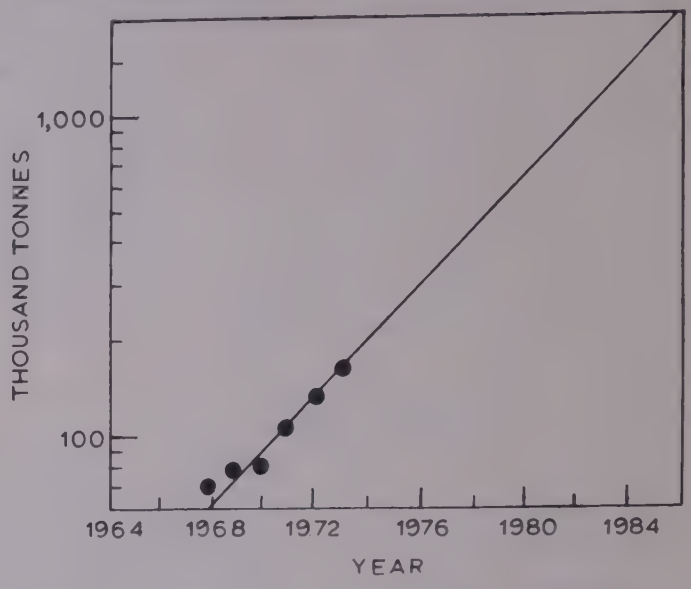

Fig. 7 — Production of cast iron castings in India

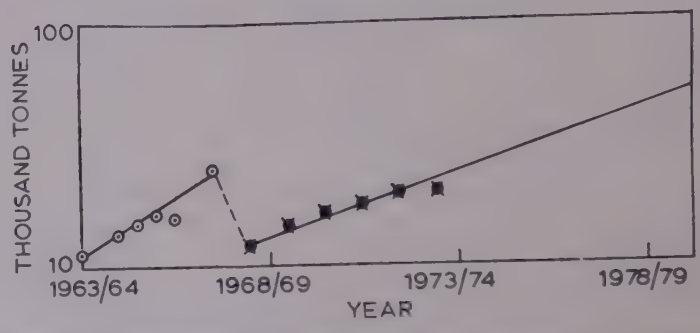

Fig. 8 — Production of malleable iron castings in India

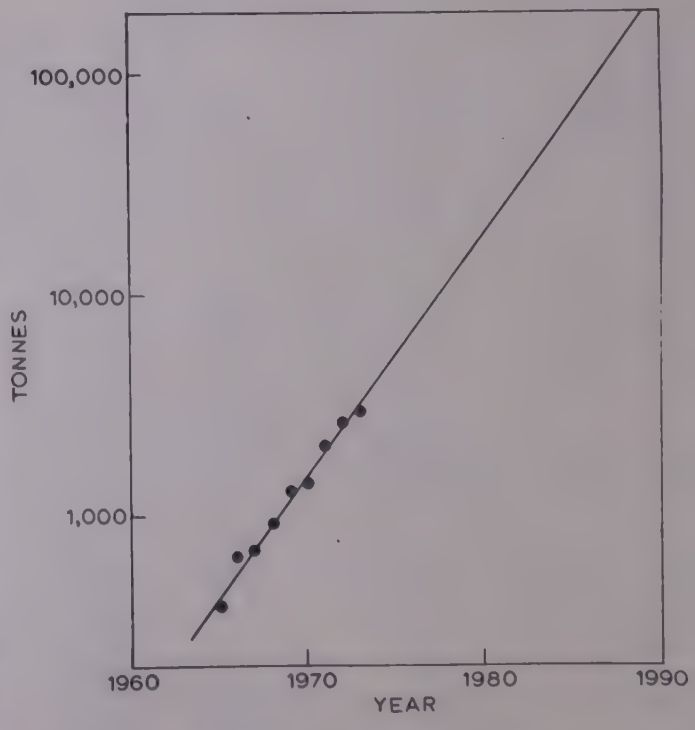

Fig. 9 — Production of SG iron castings in India

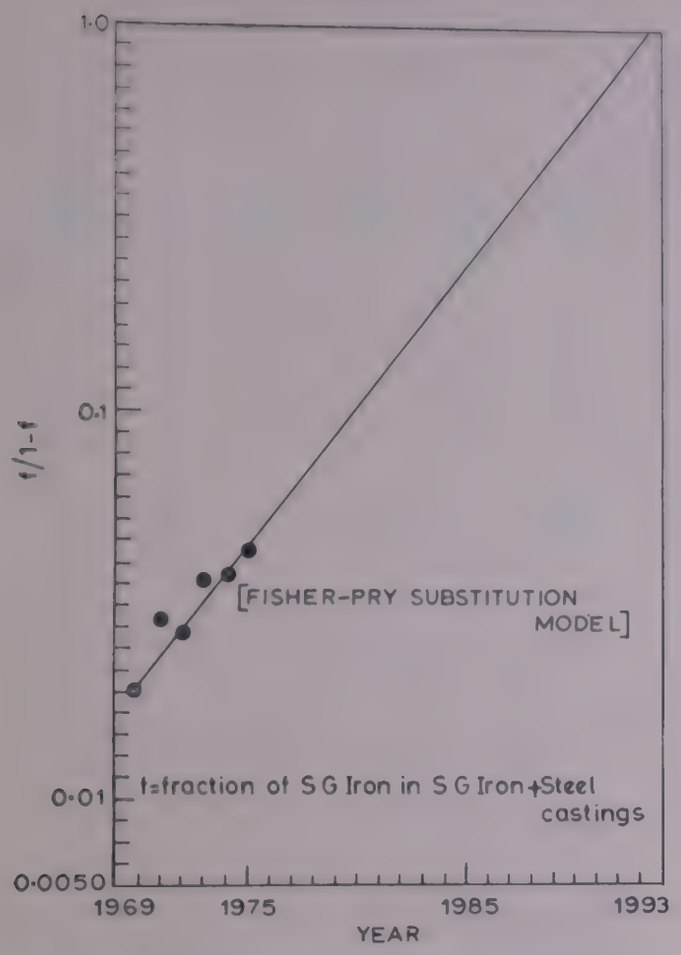

Fig. 10 — Substitution of steel castings by SG iron castings in India

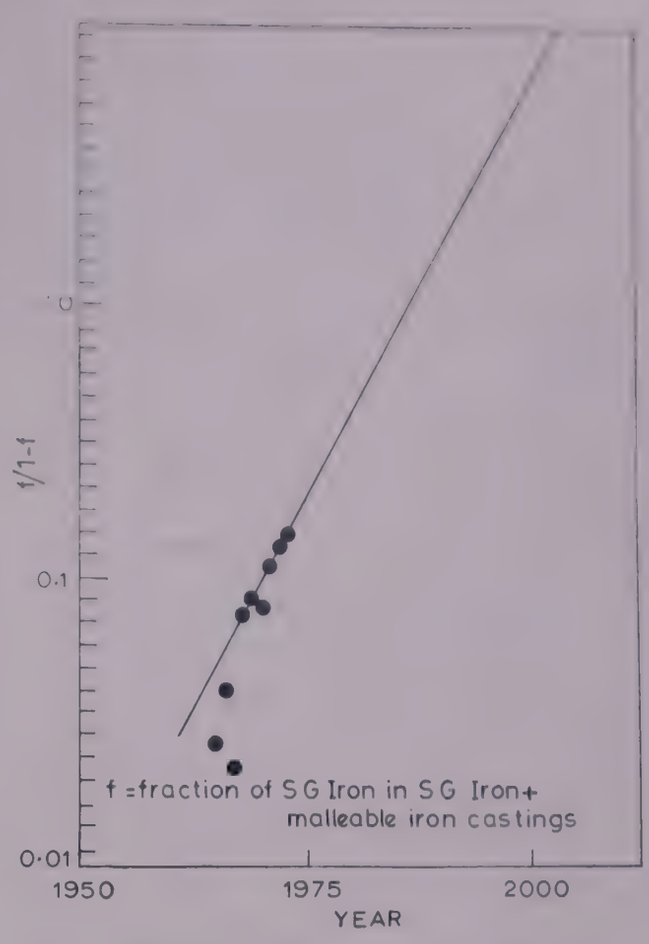

Fig. 11 — Substitution of malleable iron castings by SG iron castings in India [Fisher-pry model]

respectively by SG iron castings in India. Extrapolation of these curves shows that SG iron castings would contribute about 44% of the total SG iron and steel castings and 62% of the total SG iron and malleable iron castings produced in India in the year 1990. Combining these data with the previous forecast of the production of steel castings in the year 1990 (Fig. 5), it can be inferred that the production of SG iron castings would be around 23,00,000 tonnes. This is in fair agreement with the independent forecast obtained from Fig. 9 (i.e., 25,00,000 tonnes of SG iron in 1990) by direct extrapolation of the exponential growth curve for the production of SG iron castings in the past.

Problems of the Foundry Industries in India

In India, currently about 3·5 million tonnes of castings are produced each year in the 5000 existing units. This level of productivity is very low compared to international standards (Table 3). In view of the fact that the foundry industry is a labour intensive industry and the cost of pollution control is causing many foundries to close down in other countries, the share of production of castings from India should increase considerably towards the year 2000. In fact, India should become a prime exporter of castings. One of the reasons for failure to exploit the export potential is that there are very few foundries which can supply quality castings, and these few foundries can barely meet the internal demands. The other reasons for the low production level of the foundries are: (i) there is underutilization of capacities in many foundries due to shortage of energy and raw materials; (ii) full scale production of non-ferrous castings has not been exploited; (iii) modernization and mechanization of foundries have not been undertaken on a large scale; (iv) the knowhow and the materials for the manufacture of some essential plants, machinery and tools are not presently available in India; for example, there is a need to develop cupolas which consume less coke or perhaps no coke at all[30]; and (v) there is dearth of trained personnel.

Practical Difficulties of TF for Foundry Industry of India

So far, the growth of our foundry industry and the R & D activities has been based on intuition and interests of individuals or with a short-term profit motive. In view of the growing demand for castings by various industries, it is necessary to formulate long-range plans both in terms of R & D imperatives and the buildup of production infrastructure for the future foundry industry in India.

It is difficult to do TF for India, specially using trend extrapolation techniques, since there is lack of reliable statistics of the past performance of

TABLE 3 — CASTINGS PRODUCTION IN VARIOUS COUNTRIES*

[Values are in thousand tonnes]

Country	Grey iron	Ductile iron	Total† grey & ductile iron	Malle-able iron	Steel	Total ferrous	Copper alloy	Alumi-nium	Magne-sium	Zinc alloy	Total non-ferrous†
India (1970)	2105	17	2122	84	271	2478	40	2	NA	NA	44
Japan (1969)	4444	810	5253	419	861	6534	119	312	NA	130	561
U.S.A. (1969)	14649	1286	15935	1149	1900	18984	426	849	21	576	1903
France	2217	346	2563	103	300	2965	47	141	1	37	226
West Germany	3971	438	4409	309	417	5136	106	246	44	68	473
U.K.	3757	218	3975	232	303	4510	82	153	2	82	319
USSR (1968)	19731	‡	19731	‡	NA	19731	NA	NA	NA	NA	NA
Italy	1324	83	1407	95	158	1660	72	159	2	40	272

*Source : American Foundrymen's Society.

†Totals do not add because of rounding. Total non-ferrous also includes other non-ferrous castings.

‡Included among grey iron castings.

TABLE 4 — STATISTICS FOR RAILWAY WAGON PRODUCTION IN INDIA

Year	Source							Year	Source						
	1	2	3	4	5	6	7		8	9	10	11	12	13	14
1950-51	2900						2900	1950	2900						
1955–56	15300				15228			1955			15300				
1956–57								1956							
1957–58					16183			1957							
1958–59					11747			1958							
1959–60					8819			1959							
1960–61	11900			8200	8388		11900	1960	6900		7500				
1961–62					11375	18365		1961			10500				
1962–63					15289	23317		1962			17000				
1963–64					20417	25137		1963			19200				
1964–65					24170	27565		1964			23600				
1965–66	33500			23500	23827		33500	1965		24983	25000				
1966–67	21200			15000				1966	16000	16683	16700				
1967–68	17600			17600				1967		13383	12000				
1968–69	16500			15800				1968		14500					15248
1969–70	14900		14900				14900	1969		16000			13214		13214
1970–71			11100				11000	1970	12500	10489			9477	12500	8597
1971–72		7696	8500				8600	1971		8010		8010	7454		
1972–73		9400						1972		9721		9050			
1973–74		10100						1973		11000		10146			

SOURCE :
1. *Records & Statistics*, **22** (1971), 150.
2. *Records & Statistics*, **25** (1974), 215.
3. *Records & Statistics*, **24** (1973), 24.
4. Ref. No. 19.
5. Ref. No. 16, p. 159.
6. *Economic Times*.
7. Ref. No. 19, p. 235.
8. *Economic Times, Annual*, (1972), 97.
9. Ref. No. 18.
10. *Records & Statistics*, **20** (1968), 16.
11. *Records & Statistics*, **25** (1974), 88.
12. *Records & Statistics*, **24** (1972), 23.
13. *Records & Statistics*, **22** (1971), 213.
14. *Records & Statistics*, **23** (1971), 21.

technological parameters, such as (i) maximum weight of a single casting, (ii) cast surface finish, (iii) percentage yield of casting, and (iv) production rate.

For accurate demand forecasting, separate break up figures for sector-wise consumption of castings are necessary. For each sector, past data are needed to project the long-term requirements. Even in cases where these are available, lot of discrepancies exist. For example, data on production of railway wagons, consuming about 25% of the total steel castings produced, differ widely (Table 4). In addition, pats production figures for non-ferrous castings and their breakup are not separately available for periods earlier than 1973. For effective forecasting and planning, reliable statistics and information about growth of various technological parameters should be collected.

In view of these difficulties in obtaining past data, mainly the monitoring technique has been used for doing TF for Indian foundries.

TABLE 5 — TECHNOLOGY FORECASTS IN FOUNDRY INDUSTRY

Sl. No.	Event	Ref.
1.	Using waste heats in foundry by designing suitable heat sink and heat transfer systems for the followings: (a) Better insulation of furnace linings (b) Drying and preheating of scrap with hot effluents of casting processes (c) Raising of cupola efficiency to 60% (d) Use of heat released during solidification and cooling of castings before shake out by air preheating or steam production (e) Placing hot castings immediately after shake out in annealing or drying furnaces (f) Removing risers and gates while the casting is still hot and placing them immediately in the melting furnaces (g) Dropping hot sand on pipes carrying water for production of steam	20
2.	Shake out systems in which sand will be removed, castings cleaned and sand reclaimed, all in one unit, thus isolating noise, dirt or heat loss (closed system)	20
3.	Direct pouring of molten metal into the moulds taken to the furnaces	20–25
4.	Initiation of annealing treatment while the casting is cooled from casting temperatures, instead of letting it cool to room temperature and then annealing it	—
5.	Insulated risers and gates. In fact, runners, gates and risers should be perfectly insulated, streamlined and preformed for direct placing	20–24
6.	Use of solar energy to meet low melting point alloys like Al, Zn, Mg etc. and preheat scrap	—
7.	Village foundries using bio-gas	—
8.	Non-destructive inspection or testing of hot castings	20–24
9.	Core making by an instant curing system (hot or cold box core making machines) using self-baking cold setting or thermosetting sands	20–25
10.	Commercialization of chemically bonded self-setting sands	20–25
11.	Fluid sand process will be a common practice	20–25
12.	Squeeze casting (casting and forging) will be a commonly used process	20–24
13.	To produce sheets and wires directly from the melt	—
14.	Use of injection moulded plastic cores for low melting point alloy castings	20–25
15.	Ductile iron production will become a continuous process using low sulphur base iron and inoculation with Mg-wire	20–25
16.	Die casting of ferrous metals including SG iron will be commercialized	20–25
17.	Controlled application of ultrasonic vibrations to moulds for reproducible structural refinement during casting	20–24
18.	Increased application of investment casting process to obtain high performance castings	20–24
19.	Improvements in the mechanical properties of high alloy castings using microchills	20–24
20.	Chill solidification in permanent moulds, generally a practice for small parts and low melting alloys will be extended to large iron base parts	20–24
21.	Production of fibre reinforced eutectic alloys by directional solidification in foundries, especially for high temperature applications and air craft industries	21
22.	High speed flaskless moulding machines resulting in decreased investment and maintenance costs and also saving in handling and storage	20–24
23.	High speed automated high pressure moulding will reach the rate of 900 moulds per hour (likely in 1980 in USA)	20–24
24.	On-line full mould process with plastic injection machine, mass producing duplicate patterns in polystyrene, for immediate placement in mould aggregate	
25.	Growing role of the computer in controlling equipment, scheduling, quality control, in fact the entire casting process to be controlled automatically	20- 25
26.	Bimetal castings may be more popular to create combination of wrought and cast alloys where joining by conventional means is difficult or impossible	20–24
27.	Common use of electro-slag casting process for producing crank shafts, connecting rods, valve bodies, etc.	25–26
28.	Electromagnetic mouldless casting will be developed for commercial application	20–25
29.	Commercialization of automated vacuum shielding process	20–24
30.	Development of Ti-casting alloy compositions with better castability and improved cast properties	20–24
31.	Ferro-magnetic moulding using polystyrene pattern	21, 27
32.	Development of fumeless on-line degassing and metal filteration for non-ferrous alloys (likely in 1980 in USA)	21
33.	Automatic monitoring and control of moulding materials/binders and for checking final mould quality (likely in 1980 in USA)	21

Foundry Technology Forecasts

Various future foundry technologies listed in Table 5 have been predicted mainly on the basis of needs of the countries and intuitive thinking of experts in the field.

Forecasts 1 and 2 give techniques to conserve energy, which will be a scarce resource in the future. Already many foundries in India have either under-utilized capacities or been shut down due to power and material shortages. The foundry industry derives approximately 90% of its energy needs from fossil fuels and 10% from electricity. For achieving the objective of conserving energy, waste heats in foundry operations have to be recovered by the techniques mentioned in Forecasts 1 and 2. By 1985, the Indian foundry industry should be able to cut down its power requirement by at least 10%. In India, there is need for increasing the thermal efficiency of cupola.

Forecast 3 envisages direct pouring of molten metal into the moulds taken to the furnaces. This will save substantially on the material handling and ladle maintenance costs. Also, superheating to allow for drop in temperature during ladle pouring will not be necessary. Controlled pouring can also be achieved with this system. This technique will, however, cut down the requirements of labour and the possibility of unemployment should be weighed against the possible energy savings.

Perfectly insulated pre-cast runners, risers and gates, described in Forecast 5, will cut down large amounts of the feed metal required presently, and increase the yield of castings. This will save considerable quantities of power otherwise required for remelting large sized runners and gates.

Forecast 6 predicts the use of solar energy to melt low melting alloys. This seems to be suitable especially for tropical countries like India, where solar energy is available in abundance most of the time.

Village foundries using bio-gas, mentioned in Forecast 7, will be labour intensive and will promote small scale industries around villages.

Forecast 8 concerns non-destructive testing of hot castings. In this regard the methods to detect major casting defects while the casting is still hot, should be developed. Then defective hot castings can be immediately recycled for remleting.

Forecasts 9 and 10 describe self-baking core making systems and chemically self-setting sands. These are extremely useful for jobbing foundries, especially for the production of large castings. The fluid sand process, predicted in Forecast 11, should also be developed in India for commercial applications. These techniques will cut down the power requirements for baking the cores, annealing moulds and ramming the sand. Hot and cold box core making machines and appropriate indigenous self-setting sand should be developed.

The squeeze casting technique and production of sheets and wires directly from the melt, mentioned in Forecasts 12 and 13 respectively, will eliminate power intensive forging, rolling and wire drawing operations.

Ductile iron production in the world has a growth rate of 15% per annum. The applications of ductile iron will grow at the expense of steel, malleable iron, bronze and brass. SG iron castings can replace steel forgings in components subject to complex stressing (e.g. automobile crank shaft and connecting rod). It can also replace steel castings used for high temperature applications. In India, it would be easier to switch over to hot blast water-cooled cupolas for

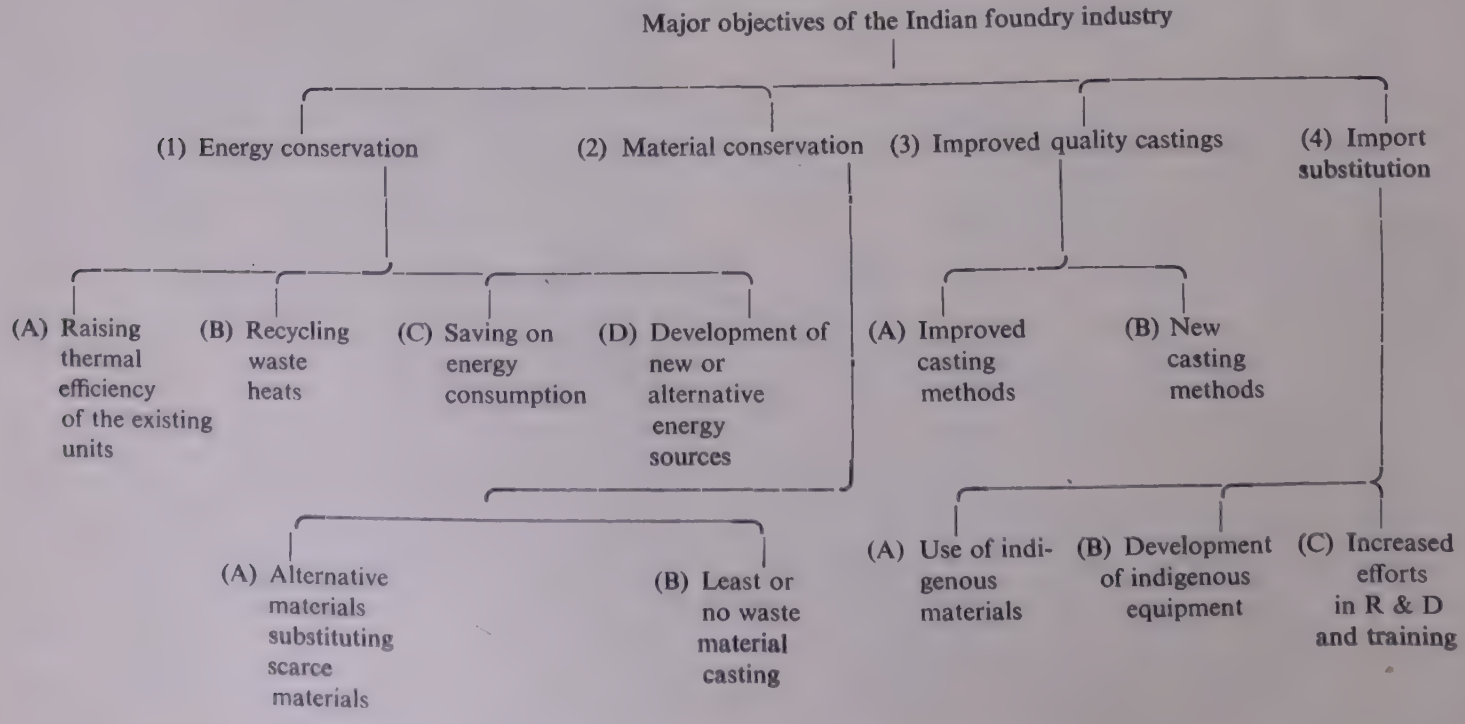

Chart 1 —Fine mapping chart for the Indian foundry industry

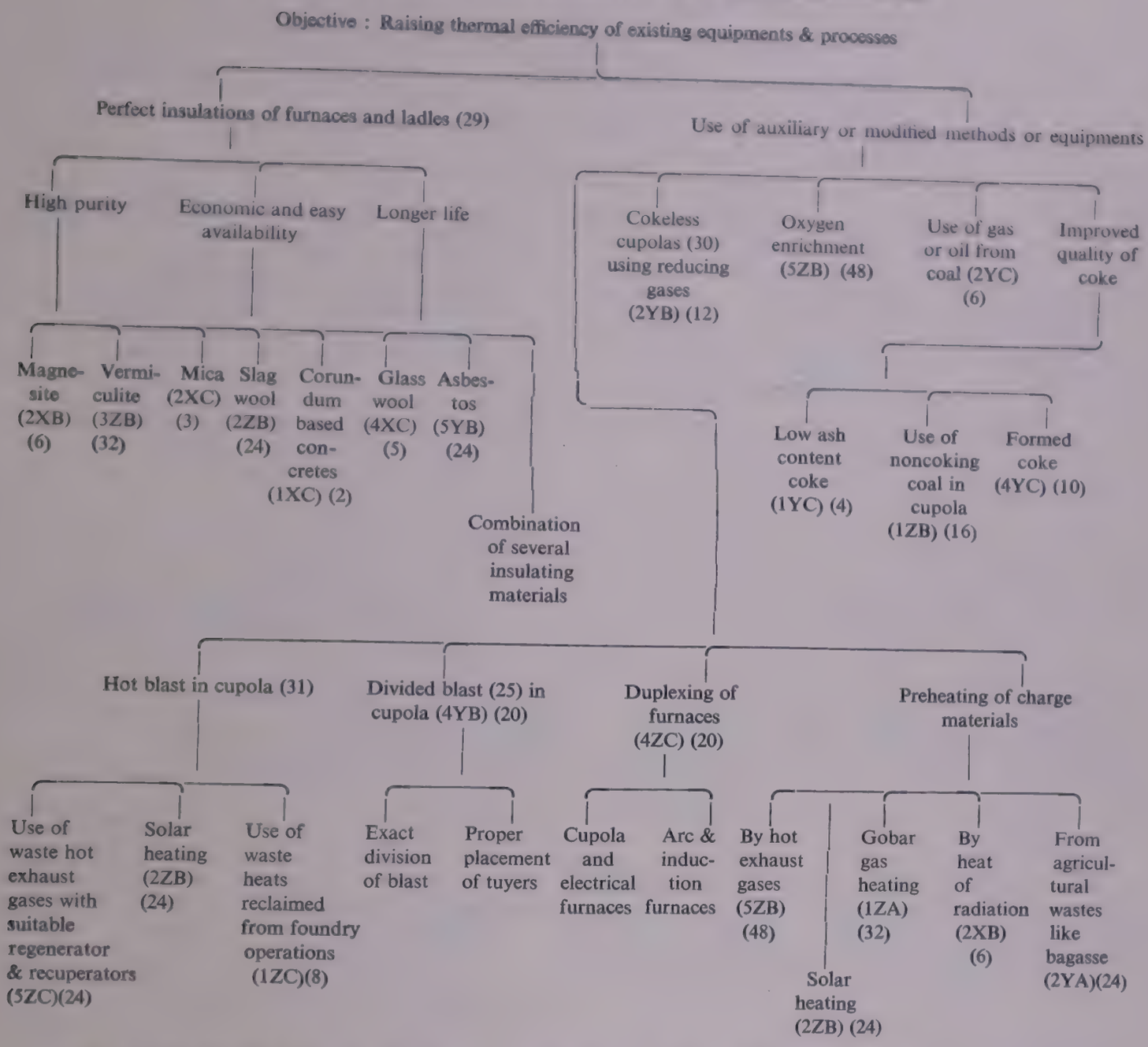

Chart 2 — Fine mapping chart for objective 1(A): Raising thermal efficiency of the existing units
[In charts 2–10, figures in the first bracket indicate score numbers assigned to the imperative and the number in the second bracket indicates the final relevance number of the imperative (*c.f.* Table 6)]

the production of SG iron. Cheaper techniques of desulphurizing pig iron, degassing and nodularizing should be found out as early as possible. In the wake of the above mentioned requirements, Forecasts 15 and 16 about continuous casting and die casting of SG iron leading to high casting yields, are of special interest for India.

Forecast 18, predicting increased application of investment casting (lost wax method) will promote the production of high melting Ni-base superalloy and Ti alloys useful in military and aircraft industries. The smooth as-cast surface finish obtained in investment casting will eliminate machining and save the recycling efforts. Development of titanium casting alloy compositions, having better castability and improved as-cast properties, mentioned in Forecast 30, is desirable in the wake of the growing demand for high temperature and corrosion resistant light metals and abundant availability of titanium in India.

Fine Mapping and Relevant Action Imperatives

Fine mapping diagrams of potential R & D inputs and production infrastructure developments necessary for Indian foundries have been derived on the basis of national needs and state of technology. The broad objectives are listed in Chart 1 and detailed break-up of these objectives is shown in Charts 2-10. It shows the action imperatives to achieve the previously stated objectives through the arrival of desirable future technologies as early as possible. A fine mapping chart identifies the major objectives, strategies and tactics as different levels of a tree. The tasks,

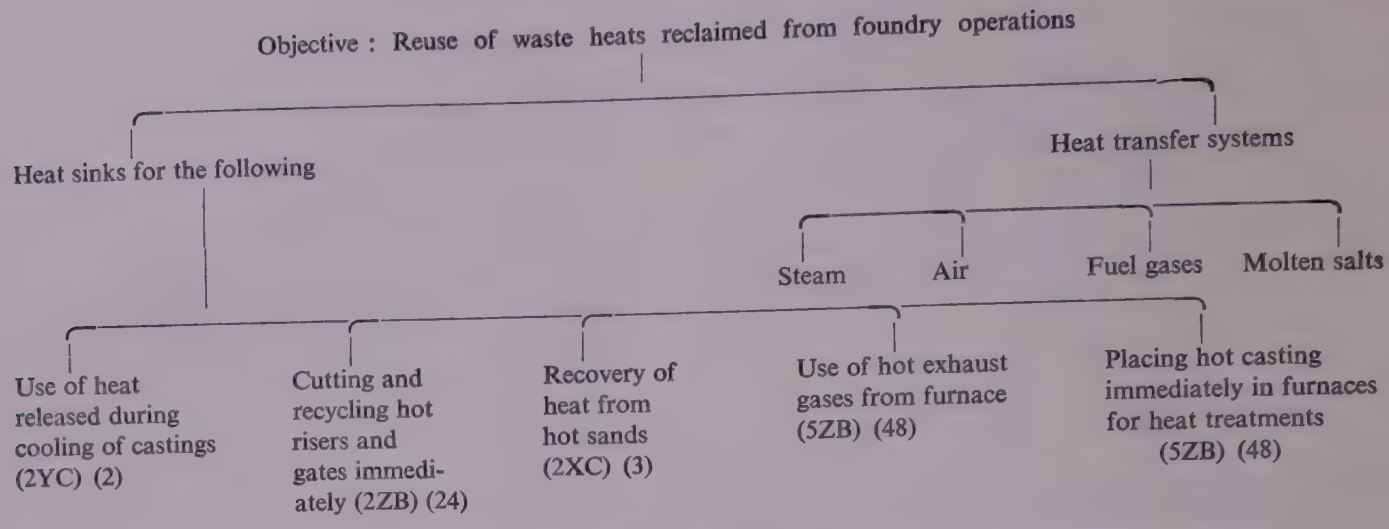

Chart 3 — Fine mapping chart for objective 1(B): Reuse of waste heat reclaimed from foundry operations

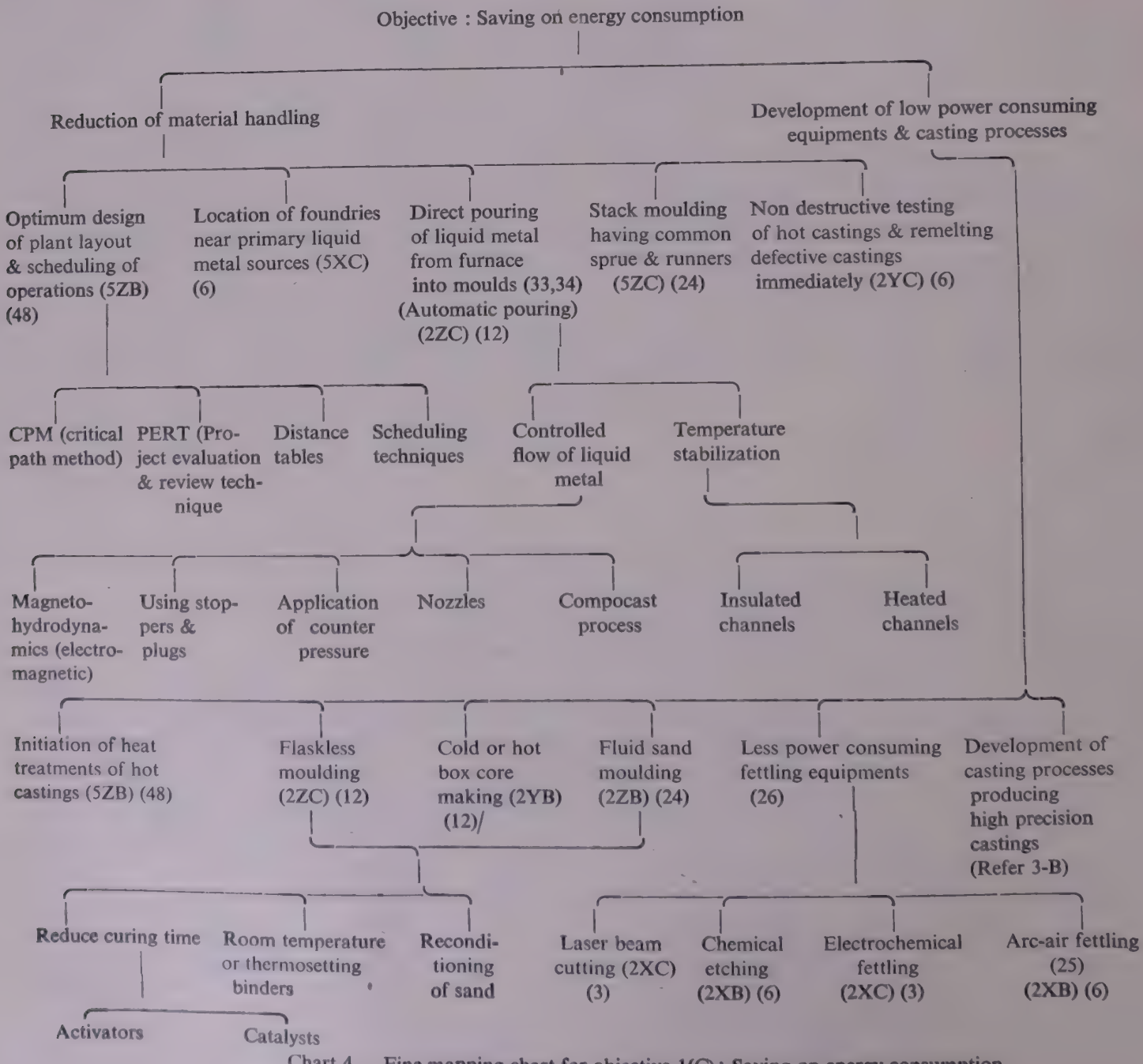

Chart 4 — Fine mapping chart for objective 1(C): Saving on energy consumption

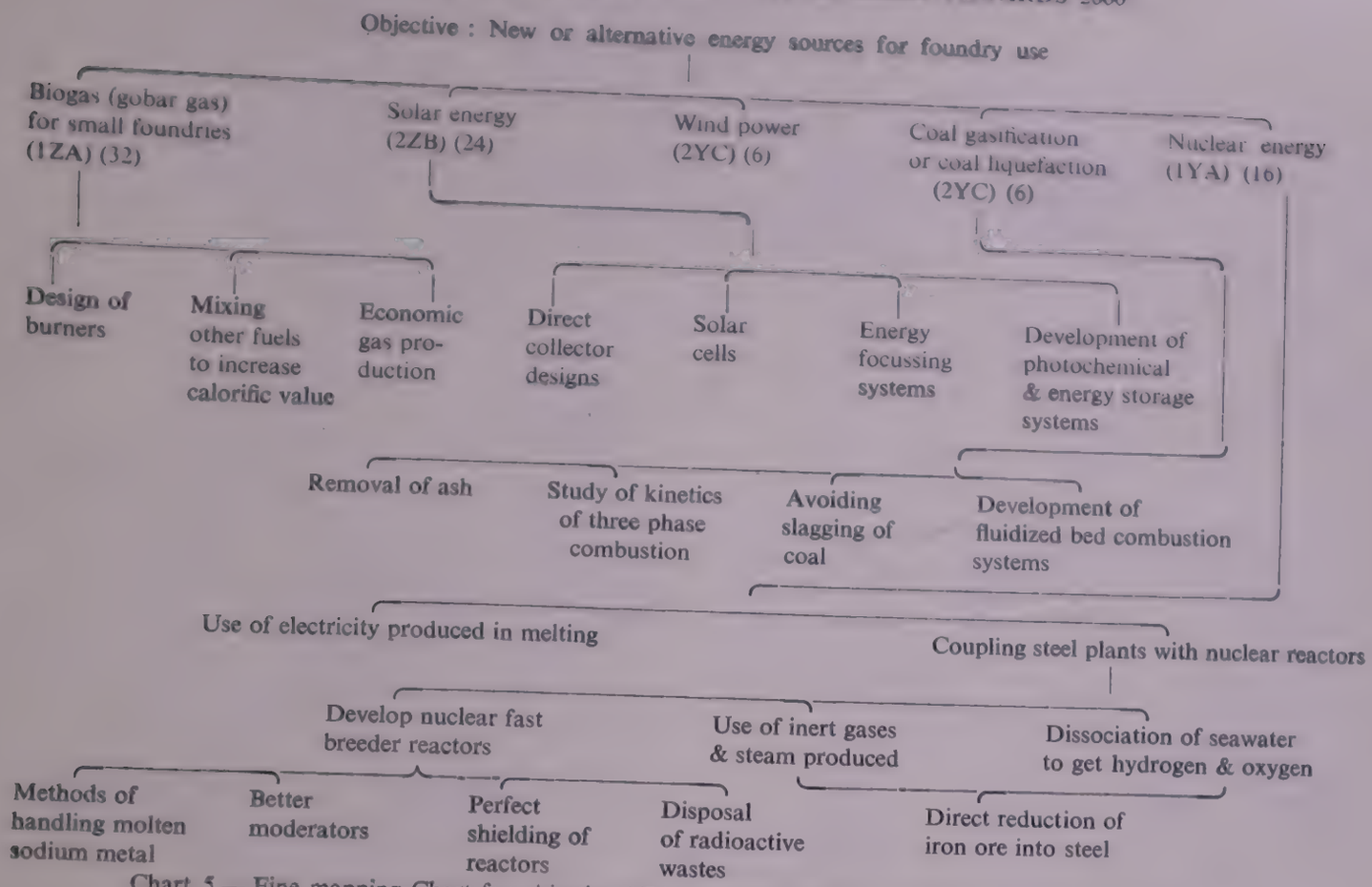

Chart 5 — Fine mapping Chart for objective 1(D): New or alternative energy sources for foundry use

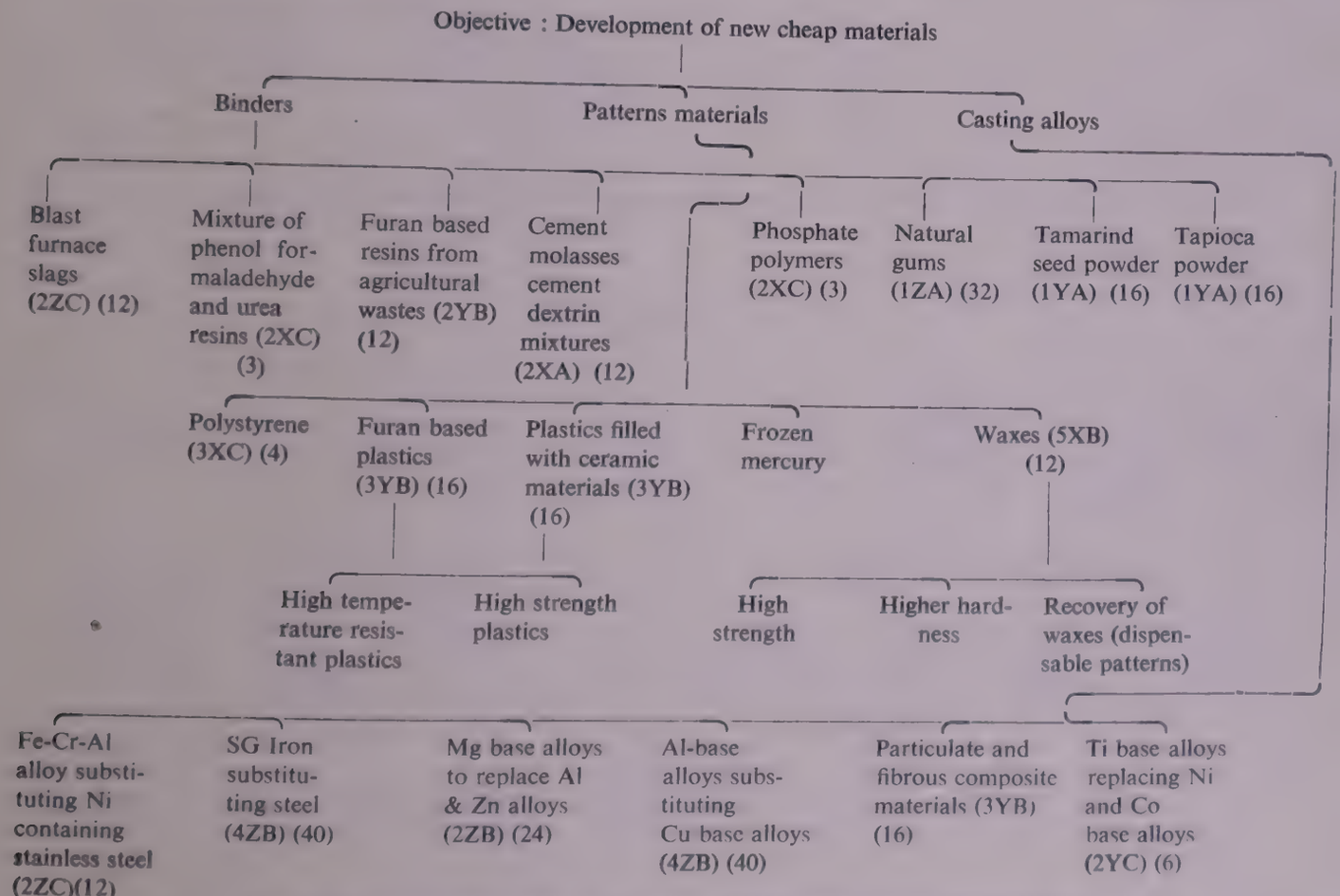

Chart 6 — Fine mapping chart for objective 2(A) : Development of new cheap materials

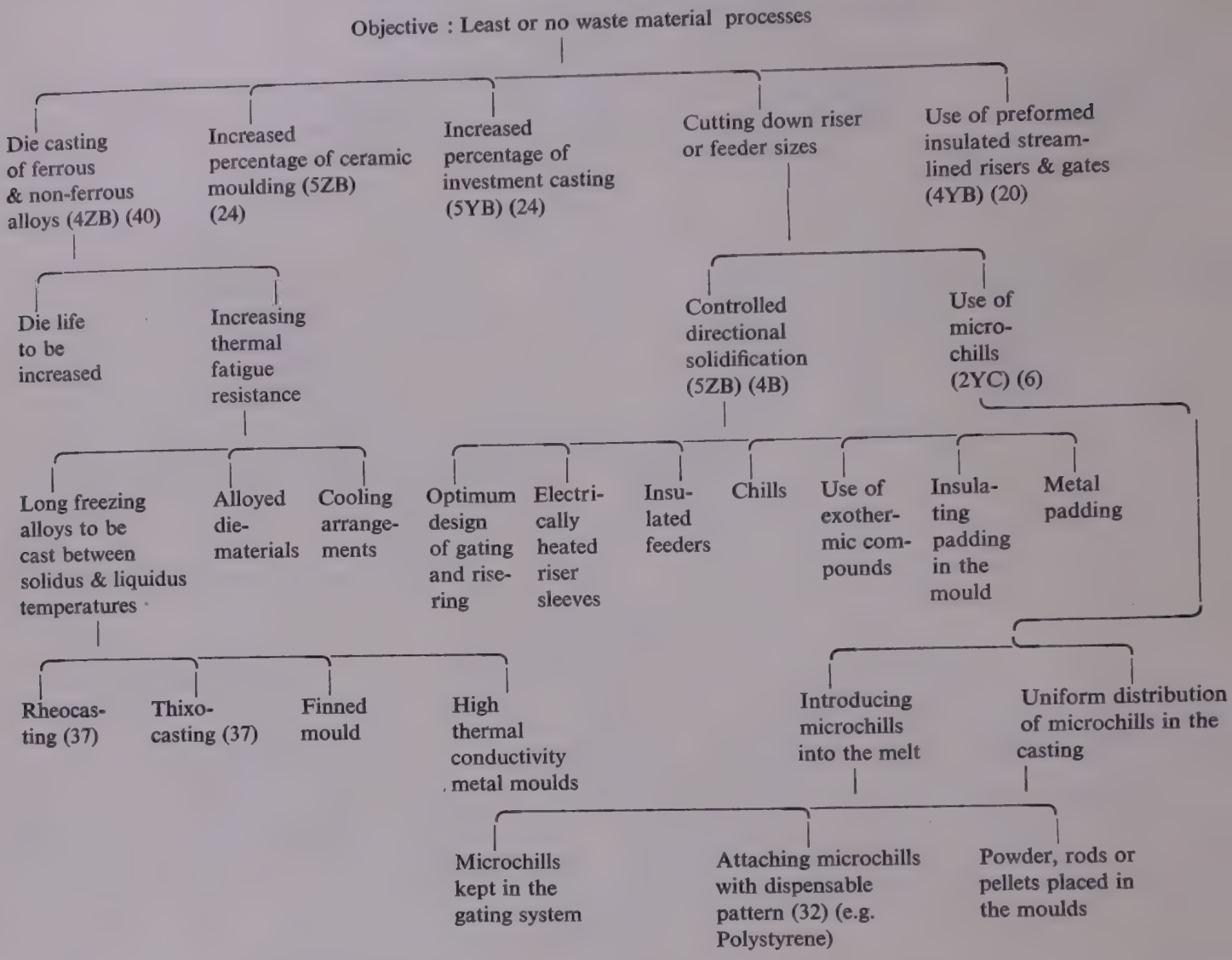

Chart 7 — Fine mapping chart for objective 2(B): Least or no waste material casting processes

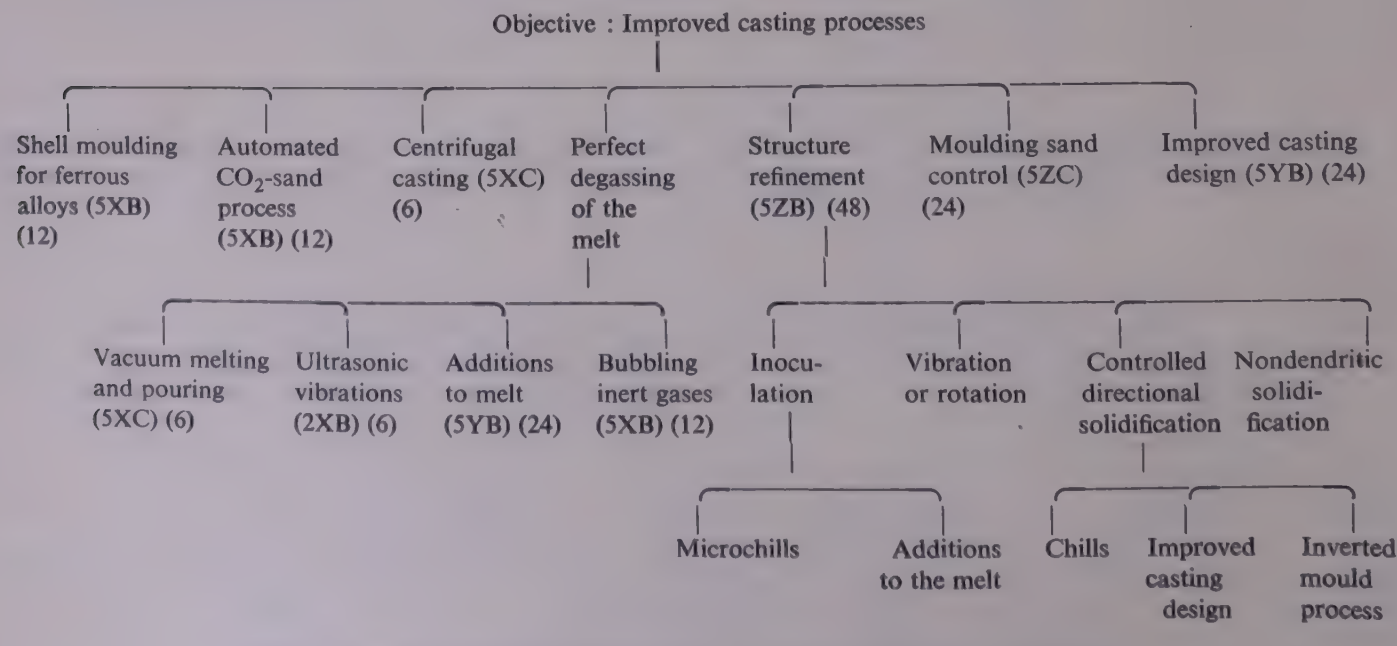

Chart 8 — Fine mapping chart for objective 3(A) : Improved casting processes

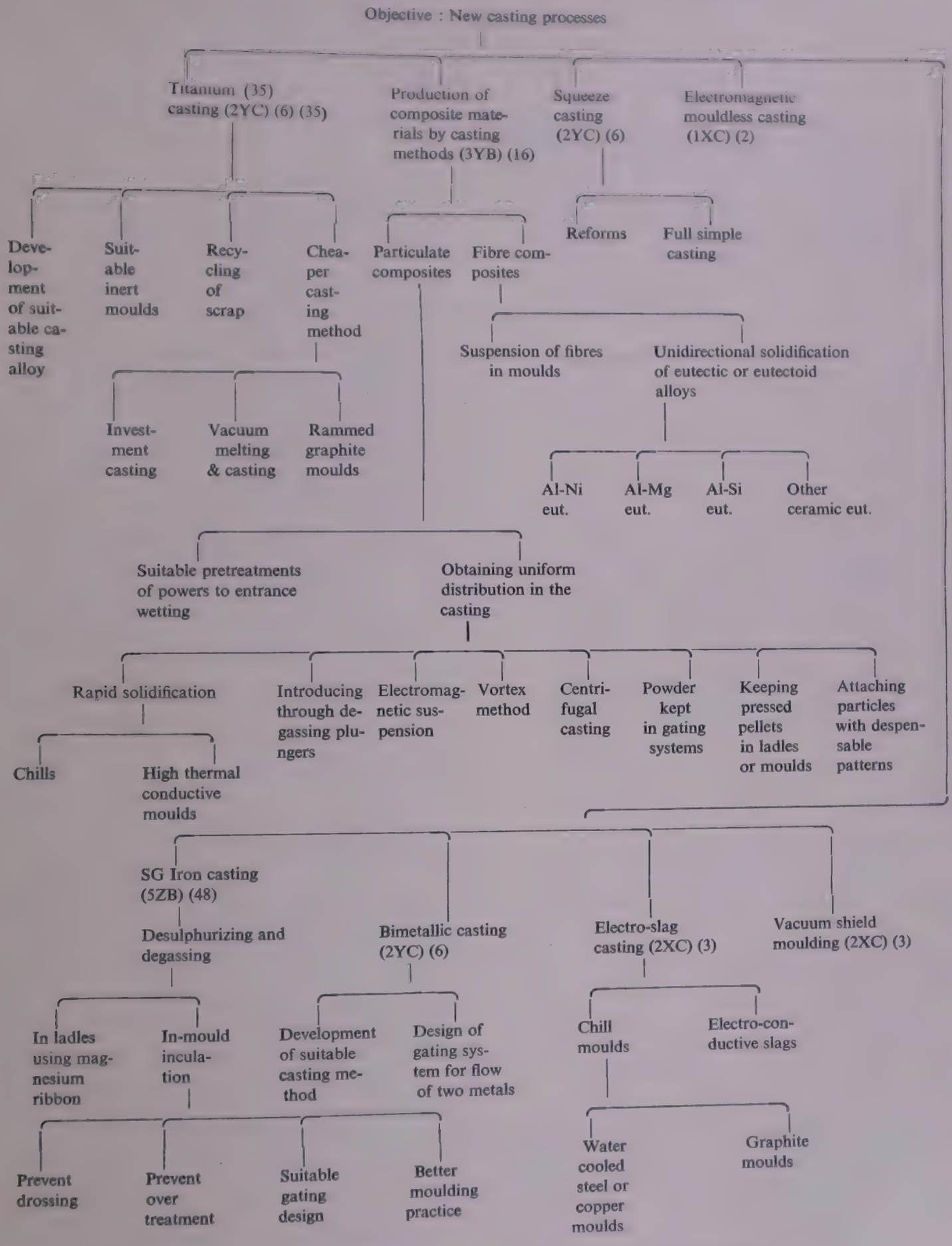

Chart 9 — Fine mapping chart for objective 3(B) : New casting processes

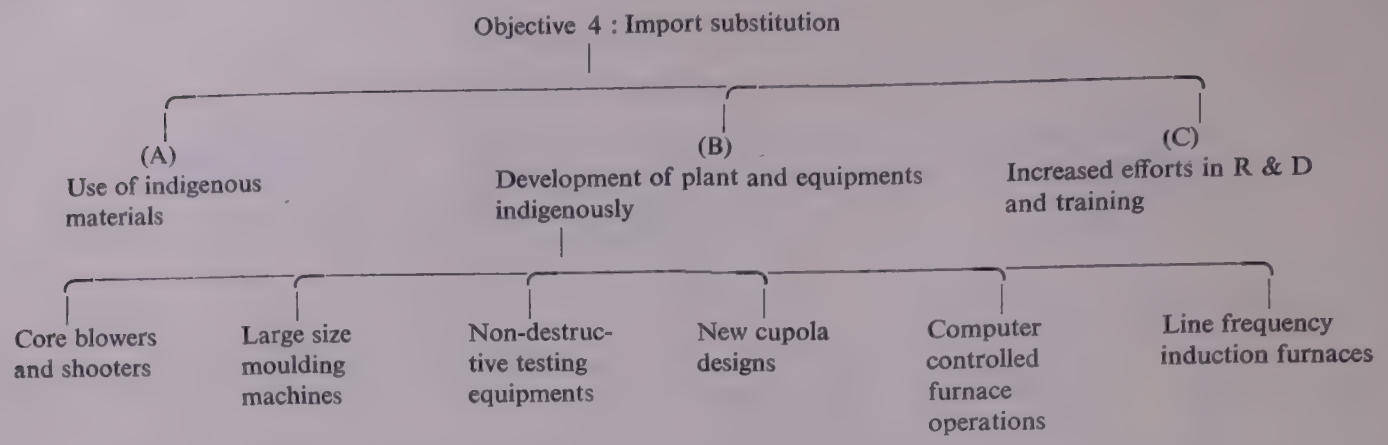

Chart 10 — Fine mapping chart for objective 4 : Import substitution

TABLE 6 — SCORES GIVEN IN THE FINE MAPPING CHART

(*i*) *State of Technology*

Number	*Description*	*Score*
0	Purely speculative	1
1	R & D effort needed know-how not available anywhere	2
2	Development effort or import of technology needed. Technology available elsewhere	3
3	Feasibility proven in India. Need to try prototypes	4
4	Prototypes proven in India. Need to set up production infrastructure	5
5	Some production units already working in India. Horizontal technology transfer within India needed	6

(*ii*) *Need and Impact*

Code Assigned		
X	Likely to have low impact—least desired	1
Y	Likely to have medium impact—desired	2
Z	Likely to have high impact—strongly desired	4

(*iii*) *Applicability*

C	Desired only for large scale adoption	1
B	Desired for small industries also	2
A	Desired specially for rural areas	4

Relevance number = State of technology score × Impact score × Applicability score

in turn, are subdivided into the required R & D thrusts or other production infrastructure developments. The priorties among various action imperatives are assigned by calculating relevance numbers. The three main criteria, considered in arriving at relevance numbers (Table 6) are: (i) state of technology (scores 1 to 6), (ii) need and desirability (scores 1 to 4), and (iii) applicability (scores 1 to 4).

The options have been classified in Table 7 as (i) high priority projects (Relevance No. 7 $\geqslant$ 40), (ii) medium priority projects (Relevance No.7 $\geqslant$ 24), and (iii) low priority projects (Relevance No.7 $\geqslant$ 12).

The judgements of the authors in arriving at final relevance numbers for various options are likely to be subjective. More quantitative methods of R & D project selection should, therefore, be used to determine priorities[22]. The relevance numbers can then be used to formulate short range, medium range and long range plans for R & D and production infrastructures for the development of future foundry industry in India. These plans should be appropriately funded (by industries and government jointly), executed and reviewed regularly. Only when such forecasting, planning and management exercises are done continuously, there can be a chance of having the right type of foundry industry in India in the future.

Scenario 2000 : Foundry Industry of India

On an average furnace charges in foundries consist of more than 80% of recycled scrap. Furnace charges are preheated either in solar furnaces or in other heating equipments using hot exhaust gases from the conventional furnaces. Furnaces are closed and compact systems in which charging, melting, furnace atmosphere, melt quality, tap to tap operations and tapping are controlled automatically by computers and control systems. In most of the foundries, electric furnaces (both arc and induction type) are used because of shortage of coking coal and increased oil prices. High speed, low and high pressure moulding machines capable of producing 300 moulds per hour are employed. Large castings are produced by fluid and self-setting sands using either furan-based resins obtained from agricultural wastes or blast furnace slag as binders. Special moulding sands, such as zircon, chromite, olivine, resin-coated sands, are used widely. Improved reclamation processes allow reuse of special sands, thus reducing the costs. Intricate cores are produced by hot or cold box core making machines. New pattern making techniques, including numerical control machining and new materials, particularly furan plastics, are employed to a considerable extent. Closed moulds are taken to the furnace for direct pouring without using ladles. Liquid metal is poured through a closed insulated

TABLE 7 — PRIORITY TECHNOLOGIES FOR FOUNDRY INDUSTRY

(I) *High priority index technologies* (Relevance number ⩾ 40)

(i) Widespread adoption of O_2 enrichment of a blast of a cupola
(ii) Widespread application of modern production planning and control tools and techniques for effective design of plant-layout, operations and scheduling
(iii) Initiation of heat treatment while casting is hot
(iv) Increased production of SG iron castings to substitute steel castings in several applications
(v) Development of aluminium alloys replacing costly copper base alloys
(vi) Increased percentage of high or low pressure die casting of non-ferrous and ferrous metals and alloys
(vii) Widespread application of controlled solidification and microchills to cut down size of risers and piping
(viii) Preheating furnace charge materials by hot effluent gases

(II) *Medium priority index technologies* (Relevance number ⩾ 24)

(i) Development of bio-gas plants for small and medium scale foundries
(ii) Use of vermiculite, slag wool and asbestos as insulating materials
(iii) Use of foundry waste gases with suitable regenerators and recuperators
(iv) Use of cheaper solar furnaces and agricultural wastes like bagasse for preheating of furnace charge materials
(v) Cutting and recycling of hot risers and gates immediately for remelting
(vi) Application of stack-moulding to reduce material movement
(vii) Widespread adoption of fluid sand process
(viii) Use of natural gums as binders in moulding sands
(ix) Increased percentage of ceramic and investment casting
(x) Perfect degassing of the melt by suitable additions to the melt
(xi) Automatic moulding sand control to eliminate all the defects attributed to improper moulding sand
(xii) Improved casting design techniques and application of controlled directional solidification to obtain better quality of castings

(III) *Low priority index technologies* (Relevance number ⩾ 12)

(i) Applications of non-coking coal and divided blast in cupolas to increase their thermal efficiency
(ii) Development of (a) furan based plastics, (b) plastics filled with abundant ceramic materials, and (c) waxes as pattern materials
(iii) Production of particulate and fibrous composites by foundry techniques
(iv) Applications of (a) Tamarind seed powder and (b) Tapioca powder as new binders
(v) Development of wind mills to provide power
(vi) Application of preformed insulated risers and gates
(vii) Direct pouring of molten metal from furnaces into the moulds
(viii) Development of self baking sand moulding techniques, using blast furnace slags, furan based resins from agricultural wastes or cement-molasses or cement-dextrin combinations
(ix) Widespread use of shell moulding and automated CO_2-sand processes for higher production rates and improved quality casting
(x) Development of in-line degassing technique to get sound castings
(xi) Development of coke-less cupolas using reducing gases

vacuum channel leading to mould openings. Filtering of liquid metals, including steel, is done using suitable filters to remove inclusions before the melt enters the moulds. Pouring rate is automatically controlled. Gates and risers are perfectly insulated and streamlined. Inoculation and addition of microchills are done either in the moulds or along with the flowing stream of molten metal in most of the castings. Controlled application of ultrasonic vibrations, rotation, and oscillation to moulds for structural refinement during casting is done on regular basis. After pouring, major casting defects are detected while the casting is still hot, and defective hot castings are sent immediately for remelting. Hot castings are cooled and fettled in an enclosure which absorbs the heat released either through blowing air or circulating water around the hot moulds. Annealing treatment is initiated while the casting is cooling from casting temperatures instead of letting it cool to room temperature and then annealing it. Used sands are crushed, refined and almost fully reclaimed. After the shake out, hot risers and gates are cut and charged immediately into the furnace.

Statistical quality control and non-destructive testing have become common practice. Also, broadened understanding of allowable defects for various applications may lead to more rational, less costly acceptance standards. Better casting techniques, such as investment casting and die casting, produce a large percentage of castings with close tolerances, reducing further machining operations. Noiseless and less power consuming fettling equipments like arc-air fettling[25] are used.

About half of the requirements of castings for aerospace, military and high temperature (e.g. turbine blades) applications are met by investment casting coupled with directional solidification. The production of SG iron is continuous and simple. Nickel-free ferritic stainless steel castings have replaced nickel containing stainless steels in many applications. The use of aluminium and magnesium alloy castings has increased considerably in transport systems, and this conserves fuel in transportation. This has led to concurrent improvement in die casting and permanent mould casting technologies, including the development of new die casting methods as thixo- and rheo-casting.

Due to acute copper shortage, copper alloy castings are used only for very special purposes where they cannot be replaced by aluminium, magnesium and steel. Solidification manipulations are regularly used to obtain controlled cast structures havings properties equivalent to those of wrought structures. It is possible to get non-dendritic solidification in many castings.

Planning of operations and layout of foundry plants and equipments are done more effectively using various modern techniques like critical path method (CPM), project evaluation and review techniques (PERT), technological forecasting and distance tables and charts. Pollution abatment systems keep foundry atmosphere clean.

Many small village foundries using gas and solar energy produce simple castings. Wherever possible, foundries are set up near primary liquid metal production centres, thus conserving energy normally consumed in remelting the ingots.

The reduced employment in foundry per unit of casting, due to automation, is more than compensated by increased overall production of castings. India has emerged as a leading exporter of castings and foundry knowhow.

Summary

The potential use of technological forecasting (TF) in planning future research, development and production in the foundry industry of India is discussed. The likely production levels of the major ferrous castings have been derived for the next 25 years, using trend extrapolation techniques. Various forecasts, done in other countries for foundry industry, are listed and evaluated in the Indian context. A fine mapping exercise indicating various action imperatives is performed. Based on relevance number method, a list of priority imperatives for R & D and production infrastructure for the foundry industry of India is given. Based on this, a desirable scenario of the Indian industry in the year 2000 is derived.

Some of the important imperatives for the Indian foundries are increased production of SG iron, aluminium - and magnesium-based alloys and die casting of ferrous as well as non-ferrous alloys. To conserve energy, development of high thermal efficiency cupolas and other furnaces, utilization of waste heats, reduction in material handling, cutting down riser sizes, and casting processes producing near zero fettled castings have to be adopted in Indian foundries.

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Thermodynamic and Transport Properties of Multicomponent Electrolyte Solutions

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DETERMINATION of the thermodynamic and transport properties of electrolytes in the presence of solutions of one or more of other electrolytes is essential for understanding the interionic forces[1]. The interionic force is a measure of the difference between the behaviours of aqueous solutions of the electrolytes and non-electrolytes. However, interpretation of the properties of these solutions is difficult because of their complicated behaviour. Nevertheless, these systems approach very close to natural biological fluids and their studies are of interest from both theoretical and practical considerations. The thermodynamic properties, such as the activity coefficient, free energy change and transport properties, such as diffusion and electrical transport of these systems have been of interest. The purpose of the present article is to review some of the equilibrium and non-equilibrium properties of these systems.

Thermodynamic Properties

The nature of electrolyte solutions has always been interpreted in terms of ionization and dissociation of salts in the solvent. The equilibrium between the ionized parts of the salt and the undissociated or unionized part is studied in the manner of law of chemical equilibrium. The equilibrium constant obtained has been related to standard free energy change as if the splitting is purely of chemical nature. This type of interpretation is qualitative and cannot be extended to multicomponent systems. Besides the free energy of mixed electrolyte solutions, equilibrium properties, such as enthalpy and volume change on mixing are of interest, particularly their magnitudes compared to those for a single component system. The one property which has been studied widely is the activity of one electrolyte in the presence of the other electrolyte using the e.m.f. technique or the isopeiestic method.

Partial molal volume — The partial molal volume has been used to study solute-solvent interactions in solutions. Dunlop and Gosting[2] developed a methodology for evaluating the partial molal volume of an electrolyte in a solution of one or more electrolytes. Consider a solution containing $q+1$ components designated by the subscripts 0, 1,.....,q. Here, zero denotes the solvent. The volume, V, of the solution containing more than one component is given by

$$V = \sum_{j=0}^{q} n_j . M_j/\rho \qquad \ldots(1)$$

where n_j represents the number of moles of the component j; M_j, the molecular weight of the component; and ρ, the density of the solution. Differentiating Eq.(1) with respect to the number of moles of the component i, at constant temperature and pressure, we get :

$$\left(\frac{\delta V}{\delta n_i}\right)_{n_{j\neq j},T,P} = \bar{V}_i = \frac{1}{\rho^2}\left[M_i\rho - 1000\left(1+\sum_{j=1}^{q}\frac{M_j m_j}{1000}\right)\left(\frac{\delta P}{\delta m_i}\right)_{m_{k\neq i}}\right] \qquad \ldots(2)$$

In deducing Eq. (2), the expression

$$m_j = \frac{1000 n_j}{n_o M_o} \qquad \ldots (3)$$

for the molality of component j has been used. Eq. (3) may be expressed in terms of molarities as :

$$\left(\frac{\delta\rho}{\delta m_i}\right)_{m_{k\neq i}} = \sum_{j=1}^{q}\left(\frac{\delta\rho}{\delta C_j}\right)_{C_{k\neq j}}\left(\frac{\delta C_j}{\delta m_i}\right)_{m_{k\neq i}} \qquad \ldots(4)$$

Making use of the following equations relating the molalities and molarities

$$m_i = \frac{C_i}{\rho - \sum_{j=1}^{q} M_j C_j/1000} \qquad \ldots(5)$$

and

$$C_j = \frac{\rho m_j}{1+\sum_{k=1}^{q} M_k m_k/1000} \qquad \ldots(6)$$

the partial molal volume, $\bar{V}_i$ becomes :

$$\bar{V}_i = \frac{M_i - 1000 H_i}{\rho - \sum_{j=1}^{q} H_j C_j} \qquad \ldots(7)$$

where

$$H_i = \left(\frac{\delta\rho}{\delta C_i}\right)_{T,P,C_{k\neq i}} \qquad \ldots(8)$$

The derivative H_i can be obtained if the density is known as a function of the solute molarities and knowing H_i one can obtain V_i for solute i from Eq. (7).

The partial molal volume in a binary system shows solute-solvent interaction and can be split[3] into two parts,

$$\bar{V}_2 = V_{in} + \Delta V \qquad \ldots(9)$$

where V_{in} is the intrinsic volume of the ion, and ΔV, the electrostriction of the solvent. In the case

of a multicomponent system, the partial molal volume of one electrolyte depends on the concentration of the other electrolytes. In such situations, the electrostriction of the solvent changes; however, the intrinsic volume of the ion remains unchanged.

The apparent molal volume, ϕ_v in the case of a solution of a single electrolyte is given by[3,4]

$$\phi_v = \frac{V - n_1 \bar{V}_1^\circ}{n_2} \qquad \ldots(10)$$

Eq. (10), in terms of densities of solutions, can be written as :

$$\phi_v = \frac{1000}{Cd_\circ}(d_0 - d) + \frac{M_2}{d_0} \qquad \ldots(11)$$

where c is the molarity of the solute; d, the density of the solution; d_0, the density of the solvent; and M_2 the molecular weight of the solute. Eq. (11) can also be used for multicomponent systems and c in Eq. (11) will represent the total solute concentration. Wirth[5] determined the apparent molal volumes of potassium chloride, bromide and sulphate in sodium chloride solution and found that concentration dependence of the apparent molal volume can be represented by the Masson's equation[6],

$$\phi_v = \phi_v^\circ + S_v\sqrt{C} \qquad \ldots(12)$$

where c represents the total concentration.

Wirth[5] found that the partial molal volumes of potassium chloride and potassium bromide vary linearly with the ionic strength, while that of potassium sulphate deviates from linearity at higher concentrations. He further found that the partial molar volumes of sodium chloride decrease at constant total ionic strength on the addition of HCl and the decrease is a linear function of the acid concentration[7]. The partial molal volumes of HCl increase at constant total concentration on the addition of NaCl and this increase is a linear function of the NaCl concentration. The partial molal volume of water was determined in the mixed solution. At a given concentration, it is maximum in pure KCl solution and minimum in pure NaCl solution. These results have been interpreted in terms of changes in the structure of water caused by ions in the solution[8].

The apparent molal volume has also been interpreted[1] for a ternary system from the apparent molal volume of individual electrolyte. Let a volume V_E of a solution containing Y_B kg of water and $Y_B m$ moles of a 1 : 1 electrolyte be mixed with a volume V_C of a solution containing Y_C kg of water and $Y_B m'$ moles of another 1 : 1 electrolyte. Let $(Y_B + Y_C) = 1$, and suppose there is an increase in volume, ΔV, on mixing. The apparent molal volume of the mixture, ϕ_v, is given by expression :

$$\phi_v = Y_B \phi_B + Y_c \phi_c + \frac{\Delta V}{m} \qquad \ldots(13)$$

where $\Delta V/m$ is the excess apparent molal volume of mixing; its value is usually very small. Young and Smith[9] found that Eq. (13) without the term $(\Delta V/m)$ is often a very good approximation. Wirth and coworkers[10] determined the excess apparent molal volume for aqueous solutions of HCl, NaCl, perchloric acid and sodium perchlorate. The excess apparent molal volume can be represented by the quantity $\Delta\phi$ and, therefore Eq. (13) can be transformed into

$$\Delta\phi = \phi_v - Y_B \phi_B - Y_c \phi_c \qquad \ldots(14)$$

It was found that $\Delta\phi$ will have either a maximum or a minimum value at $Y_B = 0{\cdot}5$; it has a small magnitude, but a larger value is obtained when the system has no common ion.

A more general, precise and simple treatment for obtaining the integral and partial molal thermodynamic quantities in a 3-component system has been developed[11] by extending the treatment of Scatchard[12] for the individual components of the solution. Gokeen[13] applied Gibb's equation for calculating the partial molar properties of components in ternary and multicomponent systems from the known partial molar property of one component. However, these equations for calculating the thermodynamic properties of ternary systems can be derived either from Gibb's free energy function or Gibbs-Duhem equation. Widerheha[14] gave a matrix formulations for estimating various properties of multicomponent systems. This concept has been applied to generate the entropy, enthalpy and free energy of multicomponent systems as functions of compositon and temperature.

Gopal *et al.*[15,17]. and Millero[18] showed that the dependence of partial molal volume at infinite dilution[19] on temperature is a very good tool for the study of ion-ion and ion-solvent interactions.

Free Energy, Enthalpy and Volume Change on Mixing

Kramer[20] derived a theory interpreting the free energy of a mixture of ions for limited concentration ranges. Berlin and Montroll[21] modified this theory. These workers gave a statistical mechanical theory of electrolytes on the assumption that (i) the ions exist as point ions, and (ii) there is a fluctuation between the ion and its unionized electrolyte. Their modification consists of a more physically accurate development of assumption (ii). The major improvement over Kramer's theory is that a partition function for the electrolyte is obtained for all concentrations, whereas Kramer's theory is not applicable beyond a limiting concentration, probably due to the non-validity of Debye-Huckel limiting law. Recently, Karapat-Yants and coworkers[22] generalized the data on heat capacity and enthalpy of mixing of 2- and 3-component solutions of group IA element chlorides and found that the additivity rule is fairly satisfactory for calculating the various thermodynamic properties.

The excess free energy of a solution of two 1 : 1 electrolyte was interpreted by Harned and Robinson[1]. Suppose that a solution consisting of Y_B kg of water and Y_B mmoles of electrolyte B were to be mixed with another solution consisting of Y_c kg of water and Y_c mmoles of electrolyte C to produce a solution consisting of 1 kg of water, Y_B mmoles of B, and Y_C mmoles of C. The excess free energy[23], G^E, is the

free energy of the mixed solution over and above that possessed by the single electrolyte solution and it is given by :

$$\frac{G^E}{RT} = W_s^{-1} \ln a_s + 2Y_B m \ln Y_B m\gamma_B + 2Y_C m \ln Y_C m\gamma_C - Y_B W_s \ln a_{s(B)} - 2Y_B m \ln m\gamma_B^\circ - Y_C W_s^{-1} \ln a_{s(C)} - Y_C m \ln m\gamma_C^\circ \quad ...(15)$$

where W_s is the molecular weight of the solvent divided by 1000, a_s the solvent activity; Y, the concentration function of the electrolyte in a mixture; γ, the activity coefficient on molal scale; and γ°, the activity coefficient of an electrolyte in its own solution. Eq. (15) contains a term $2m$ ($Y_B \ln Y_B + Y_C \ln Y_C$) which will occur even if the solutions are ideal and it is not considered part of the excess free energy. It is found that for KCl-NaCl system, the maximum excess free energy is found when the solutions of B and C are mixed in equal amounts, $Y_B = Y_C = 0{\cdot}5$, and then it is given by :

$$G^E = \tfrac{1}{4} RTm^2 (Q_B + Q_C) \quad ...(16)$$

where m is the total molality and $Q_B = \ln 10\alpha_B$; $Q_C = -\ln 10\gamma_C$ and α_B, α_C express the variation of the activity coefficient of an electrolyte with change in the electrolyte composition at constant total molality, m.

Now if the excess free energy of a mixture is given by the simple expression.

$$G^E = Y_B Y_C m^2 RT (Q_B + Q_C) \quad ...(17)$$

the excess enthalpy of mixing is given by :

$$H^E = -Y_B Y_C m^2 RT \frac{\delta}{\delta T} (Q_B + Q_C) \quad ...(18)$$

and the excess volume of mixing is given by :

$$V^E = Y_B Y_C m^2 \frac{\delta}{\delta P} (Q_B + Q_C) \quad ...(19)$$

Excess free energy cannot be measured directly; experimentally only the partial molal free energy of one component can be determined and using the Gibbs-Duhem equation, the excess free energy is obtained. The excess enthalpy of mixing is determined directly; its accuracy depends on the precision with which small heat changes can be measured.

Wu *et al.* [24, 25] obtained values of enthalpy change for the mixing of many pairs of electrolytes at a total molality of $m = 1$. They obtained enthalpy changes for M_2Cl-M_1Cl, where M_1, M_2 represent hydrogen or an alkali metal ion. Their results can be expressed in the form :

$$H^E = Y_B Y_C m^2 RT [h_0 + h_1 (Y_B - Y_C)] \quad ...20)$$

If $h_1 = 0$, the plot of H^E against Y_B is symmetrical with a maximum (or minimum) at $Y_B = 0{\cdot}5$. The value of h_1 is very small for these systems and consequently the maximum value of H^E for all these system is found close to $Y_B = 0{\cdot}5$. The excess enthalpy of HCl-NaCl system appears anomalously high, but there is a trend from comparatively small positive values to numerically large but negative values as the molecular weight of the salt increases.

A correlation can be seen here with the water structure-promoting and structure-breaking concept of Frank *et al.* [26,27]. Hydrogen, lithium and sodium ions promote the structure of water in the order : $H^+ > Li^+ > K^+$. Potassium ion has little effect and it promotes both the effects more or less equally; rubidium and cesium ions are, however, strong structure breakers, particularly the cesium ion. This order is also the order of ionic hydration and hydration is one form of structure making. Hydrogen and lithium ions are highly hydrated and cesium ion is probably not hydrated at all. The same trend[28] can be found in respect of the effect of ions on the viscosity of the solution and it can be expressed as :

$$\eta/\eta_0 = 1 + AC^{1/2} + BC \quad ...(21)$$

where η and η_0 are the viscosities of the solution and the pure solvent respectively. The β-coefficient is positive for hydrogen, Li, and Na ions which influence structure formation, but it is negative for K, Rb and Cs ions.

If both the electrolytes are structure makers, such as HCl and LiCl, the enthalpy change is positive, but if one is a structure promotor and the other is a structure breaker, viz. HCl-CsCl, the effect is negative. If both are structure breakers, the effect is positive; for example, in case of RbCl-CsCl, $H^E = 0{\cdot}38$ cal kg^{-1}. This shows that the effect is positive if both the electrolytes are of the same type and negative if they are of opposite types. It is, however, smaller if both are structure breakers or structure promotors.

Another feature of these results is that they seem to be little dependent on the nature of the anion : thus, for LiCl-LiBr system, the enthalpy change is only 0·81 cal kg^{-1} and it is almost same in the case of LiCl-NaCl system ($H^E = 21{\cdot}15$ cal kg^{-1}) and LiBr-NaBr system ($H^E = 20{\cdot}67$ cal kg^{-1}). These comparatively high, positive values are consistent with structure formation and suggest that it is the cations rather than the anions which influence the structure of water, whether it be promotion or breakage of structure.

The influence of the structure of water was also studied by Wood and Anderson[29]. They found negative values for H^E when potassium fluoride was mixed with potassium chloride (or bromide) solutions. Judging from the viscosities of their solutions, chloride and bromide ions are weak structure breakers, whereas the self-diffusion of water in fluoride solution suggests that the fluoride ion is a strong structure promoter[30]. This is expected from the small size of flouride ion. Thus, fluoride and chloride (or bromide) ions are opposite in type and, therefore, a negative enthalpy of mixing is expected. The acetate ion is also a structure promoter partly because of the hydrophobic nature of the methyl group and partly because of the charge density around the oxygen atoms. For KBr-CH_3COOK mixtures, H^E is large and positive, and it would seem that the rule that H^E is positive for mixing of like-type of ions holds even when their structure promoting ability is for different causes.

Activity Coefficients

If a solution containing one solute is ideal at all concentrations, the activity coefficient of the solute is unity at all concentrations. On mixing two such ideal solutions, the activity coefficient of each solute will be unity, if there is no interaction. Experimental measurements of activity coefficients of a multi-component electrolyte solution will be an excellent measure of interactions in these solutions. In practice one salt does influence the activity coefficient of the other[31,33].

Free Energy of Solutions

The total free energy of a solution can be written as

$$G = G^{id} + G^{el} + G^{sol} + G^{E} \qquad ...(22)$$

where G^{id} is the free energy of the ideal solution : and G^{el} is taken to mean the free energy resulting from interionic forces of the Debye-Hückel type. G^{sol} is the free energy due to ion-solvent interaction, and G^{E} is due to ion-ion interaction apart from the Dubye-Hückel type interaction. The contribution of the interaction of the ions and the solvent molecules can be written as[34] :

$$\ln \gamma_B^{solv} = -h_B/\nu_B \ln a_S - \ln[1 + W_S(\nu_B - \bar{h}_B)m_B] \qquad ...(23)$$

where $\bar{h}$ is a solvation or hydration number. Eq. (23) can be expanded in a power series as :

$$\ln\gamma_B^{solv} = Am_B + Bm_B^2 + Cm_B^3 \qquad ...(24)$$

Here the term in the first power of m_B is always dominant. The contribution of ion-ion interaction is also found to take a similar form in which the term m_B is again dominant. It is, therefore, difficult to separate ion-solvent from ion-ion interaction, and so it is usual to combine G^{solv} and G^{E} in one term :

$$G = G^{id} + G^{el} + G^{E} \qquad ...(25)$$

The free energy part defining the solute-solvent interaction, G^{E}, is related to activity coefficient as :

$$\nu_B \ln \gamma_B = \nu_B Z_1 Z_2 \ln \gamma^{DH} + \frac{1}{RT}\left(\frac{\delta G^E}{\delta n_B}\right)_{n_S, n_C} \qquad ...(26)$$

where

$$\ln\gamma^{DH} = -\frac{\ln 10 A I^{1/2}}{1 + pI^{1/2}} \qquad ...(27)$$

and A is the constant of the solvent ; I, the ionic strength; and p, the Debye-Hückel parameter. Therefore, the evaluation of the activity coefficient of one solute in the multicomponent system can give an idea about the derivative, $(\delta G^E/\delta n_B)_{n_s n_c}$, which, in turn, shows solute-solvent interaction with respect to the salt whose activity coefficient is determined and hence it can throw light on the nature of the salt from the viewpoint of the structure making/breaking characteristics.

Ion-ion interactions in dilute solutions of two electrolytes were studied by Guggenheim[35,36] and Scatchard[37]. These workers assumed that the excess free energy due to ion-ion interaction is given by

$$\frac{G^E}{RT} = \frac{1}{2}\sum \frac{n_j\, n_k\, B_{jk}}{n_s + \Sigma\, n_j} \qquad ...(28)$$

where B_{jk} is a measure of the j (ion) -k(ion) interaction. Here, they introduced the Bronsted's principles of specific interaction according to which interaction between like charged particles is weak and the summation in Eq. (28) is to be made only over pairs of oppositely charged ions.

If the two electrolytes B and C have an ion in common, e.g. HCl and CsCl, the activity coefficient for the two electrolytes is given as

$$2\ln\gamma_B = 2\ln\gamma_B^{\circ} + (B_{23} - B_{12})\, Y_C m \qquad ...(29)$$

and $$2\ln\gamma_C = 2\ln\gamma_C^{\circ} + (B_{12} - B_{23})\, Y_B m \qquad ...(30)$$

where γ° is the activity coefficient of an electrolyte in its own solution. Thus, the properties of the mixed electrolyte solution are determined solely by the properties of the single electrolyte solution, since B_{12} and B_{23} are characteristics of solutions of B and C respectively. The logarithm of the activity coefficient of each electrolyte is a linear function of the composition of the electrolyte mixture and the plots have slopes of equal magnitude but opposite in sign.

Transport Properties

If an equilibrium system is changed to a non-equilibrium state by some external or internal disturbance, one of the state parameters becomes a function of position. When the disturbance is removed, an irreversible decay process occurs spontaneoulsy, and the system advances through a series of non-equilibrium states until equilibrium is reached. The decay process is a transport process, the name arising because some quantity is transferred through the system as the system attempts to make all parameters independent of position. Spatial dependent of the parameters is, therefore, associated with a flux of some kind. The non-equilibrium systems of interest can be described by the state variable and their linear derivatives. It is then logically assumed that the relation between a gradient X and its conjugate flux j is linear[38]; thus

$$J = LX \qquad ...(31)$$

If Eq. (31) is valid, the system is said to be close to equilibrium at all times. The rate at which the system approaches equilibrium is determined by the proportionality constant of Eq. (31), i.e. by the transport coefficient L_i.

In a diffusing or conducting system, the flow of an ion should be proportional to the gradients of its intensive properties and would be uninfluenced by the presence of other distant ions. On this basis, the Nernst-Hartley (N-H) equation for the diffusion coefficient in the limiting region is written as :

$$D = D^{\circ}\left(1 + \frac{d\ln\gamma_{\pm}}{d\ln C}\right) \qquad ...(32)$$

where D° is the limiting value of D at infinite dilution; γ, the mean molar activity coefficient; and C, the concentration of the solute in mol-l.

So far, the vectorial transport properties have been shown to be predicted and explained only in terms of molecular theories, which were necessarily confined to very dilute solutions. With the advent of irreversible thermodynamics, a second macroscopic framework necessary to explain the phenomenological theory of the vector transport properties, such as conductance and diffusion was developed[40-44]. It gives a natural and completely general description of irreversible processes in terms of the linear transport coefficient. Clearly, these are more fundamental than the common transport coefficient for special cases, such as equivalent conductance or diffusion coefficient, which turn out to be combinations of the different transport coefficients, l_{ij}. This description fully takes into account the effect of the flow of one constituent on the properties of all the other constituents.

Any vector transport process, no matter how complex, can be completely characterized once the values of l_{ij} are known as functions of temperature, pressure and concentration. The more important parameteres are the cross-coefficients l_{ij} $(i \neq j)$; they directly represent the ionic interactions and their exclusion is responsible for the non-validity of simplified electrolyte theories.

Although the l_{ij} are fundamental and give a new insight into the behaviour of electrolytes, their values cannot be known easily and little is known about their concentration dependence. Miller[45, 46] derived equations relating these fundamental transport coefficients in terms of experimentally measured quantities for binary and ternary systems. Rigorous expressions for the conductances (Λ) transference number (t_i), and solvent fixed thermodynamic diffusion coefficients $(L_{ij})_0$ in terms of the solvent fixed ionic transport coefficients, l_{ij}, for a ternary system $C_{r_{1c}}\ A_{r_{1a}}\ - D_{r_{2c}}\ A_{r_{2a}} - H_2O$ are given by Miller[45, 46] as :

$$\Lambda = \frac{1000F^2}{N} \sum_{k=1}^{3} \sum_{l=1}^{3} Z_k\, l_{kl}\, Z_l \qquad \ldots(33)$$

$$t_k = \left[Z_k \sum_{l=1}^{3} Z_l\, l_{kl} \right] \Big/ \alpha \qquad \ldots(34)$$

where

$$\alpha = \Lambda N / 1000F^2$$

and

$$(L_{ij})_o = \frac{\sum_{k=1}^{3} \sum_{l=1}^{3} \frac{Z_k\ Z_l}{r_{ic}\, r_{jc}} (l_{ij}\ l_{kl} - l_{il}\ l_{kj})}{\sum_{k=1}^{3} \sum_{l=1}^{3} Z_k\ l_{kl}\ Z_l} \quad (i,j = 1, 2) \qquad \ldots(35)$$

where Z_i are the signed valencies of the ions r_s the stoichiometric coefficients for ionization, and N, the normality. A simultaneous solution of these three equations can be used to predict the concentration dependence of l_{ij}. Paterson *et al.*[47-48] applied the methodology of Miller to binary systems CsCl – H_2O, RbCl – H_2O and used it to calculate the concentration dependence of l_{ij}. They combined the experimentally measured conductances and diffusion coefficients with the literature values of activities and transport numbers to provide a completely irreversible thermodynamic analysis for aqueous RbCl solutions in the concentration range 0·25-3·0 *M*. Similarly, they combined the experimentally measured conductances with literature values of transport numbers, diffusion coefficients and activity coefficients for CsCl. The variation in the values of l_{ij} with concentration has been shown by Pikal[49] to be affected by ion association.

Conductance Studies

Electrolytic conductance is one of the most direct evidences for the existence of ions in solution. In solutions of a single electrolyte, its variation with concentration is an important way of studying ionic equilibria. Conductance measurement is one of the very few available experimental methods with which the formation of ion pairs and complexes in solutions of electrolytes can be studied. In a symmetrical electrolyte, MA, any ion pair formed will be effectively uncharged and will not contribute[50-54] to the conductivity of the solution.

The conductance of mixtures of electrolytes and the way in which they depart from the direct addition of the component conductance have also been used to provide information about ion association. This procedure of predicting ion association in mixtures of electrolytes suffers from the serious drawback that departure of the observed conductance from additivity may be caused not only by direct ionic interaction but also by the effect of the added ions upon the relaxation time contribution[55]. For this reason and due to the fact that no proper theory exists for conductance in mixed electrolytes, very little work has been done in this field.

Kell and Gordon[56] derived a series of equations for the equivalent and ionic conductance in mixtures based on Onsager-Fuoss theory[57]. One of these equations is :

$$\lambda_i = \lambda_i^\circ + \nu_i\, \lambda_i^\circ\, J^{1/2} - \sigma\, J^{1/2} \qquad \ldots(36)$$

and it represents the conductions for a solution containing three univalent ions. The coefficients ν_i and σ are defined as

$$\nu_i = 1.98 \times 10^6 (2^{1/2})\, Z_i\, (1 - H^{1/2})_{is}\, r_s / (DT)^{3/2}$$
$$\sigma = 29.15\ (2^{1/2}) / (DT)^{1/2}\, \eta \qquad \ldots(37)$$

where Z_i is the sign of the charge of the ion : D, the dielectric constant; and η, the viscosity of the solvent at temperature T; the subscript i indicates summation over the three ions and the vectors r are $+1$ for the cation and -1 for chloride. For a single binary electrolyte, the matrix product $Z_i(1 - H^{1/2})_{is}\, r_s$ becomes 0.29289. The change of conductance of the ion in

the mixture as compared with its conductance in binary solution is ascribed to the change in the time of relaxation coefficient, ν_i. To compute for a given μ (ion fraction of K in the mixture of KCl and LiCl), the matrix elements h_{ji}, one requires the nine quantities $W_{ji} = \lambda_j^\circ/(\lambda_j^\circ + \lambda_i^\circ)$, where $\lambda_x^\circ, \lambda_{Li}^\circ$ and λ_{Cl}° are known. From these one computes the matrix product and one writes :

$$Z_i (1 - H^{1/2})_{is}\, r_s = Z_i (a r_i + b h_{is} r_s) \qquad \ldots(38)$$

The difference between this quantity and 0.29289 when multiplied by $(0.2289/0.2929)\, \lambda_i^\circ\, j^{1/2}$ gives the change in ionic conductance in the mixture as compared with that in binary solution at the same total ionic strength, J. From the resulting ionic quantities, $\triangle\Lambda$ may be calculated. In the case of cations, the differences between the calculated and observed values of $\triangle\Lambda_i$ are greater than the experimental error. However, for the chloride ion, the discrepancy is slightly greater than the apparent precision of the chloride data and the observed values of $\Delta\Lambda_i$ are numerically greater than the estimated values. The assumptions in the theory of the mixture effect are essentially those inherent in the limiting law for binary electrolytes, and for the alkali chlorides at these concentrations, the limiting law overestimates the effect of ionic interaction. It is assumed that the higher order terms are the same in the mixture as in binary solution and, therefore, it is seriously in error and no theory of mixture effect for the higher order terms is available.

Most of the data available for the ternary systems are for the following mixtures : (1) NaCl—KCl—H_2O; (2) LiCl—KCl—H_2O; (3) KCl—HCl—H_2O; and (4) NaCl—HCl—H_2O.

Data for NaCl—KCl mixture are available at high concentration (0.1-4M) and Miller[45] has shown that the conductance at lower concentrations can be predicted correctly by the mixture rule.

No work has been reported on systems higher than ternary mixtures. Recently, an attempt has been made in this laboratory to obtain data on the conductance of the quaternary system LiCl-NaCl-KCl-H_2O at different temperatures and at different combinations of concentrations. Values of specific conductance were calculated for the following systems :

LiCl (0.01 N) + NaCl (0.01 N) + H_2 (System A)
LiCl (0.01 N) + KCl (0.01 N) + H_2O (System B)
NaCl (0·01 N) + KCl (0·01 N) + H_2O (System C)
LiCl (C) + NaCl (0·01 N) + KCl (0·01 N) + H_2O (System I)
LiCl (0·01 N) + NaCl (C) + KCl (0·01 N) + H_2O (System II)
LiCl (0·01 N) + NaCl (0·01 N) + KCl (C) + H_2O (System III)

at 30°C, 40°C and 50°C for C varying from 0·001 N to 0·01 N. The specific conductance χ_i of ith salt whose concentration is changing in the systems I, II and III is calculated using the relation :

$$\chi_i = \chi_{n+i} - \chi_n \qquad \ldots(39)$$

where χ_{n+i} is the specific conductance of the systems I, II and III containing three electrolytes and χ_n is the specific conductance of the systems A, B, and C. On plotting χ_i against the concentration of the salt i, it is found that a straight line fits the data at all temperatures and that the specific conductance increases with increase in the concentration of the component i. Also, if we plot χ_{n+i} against the concentration of the component i, a straight line fits the data. This plot was extrapolated to $C_i \to 0$ and it was observed that χ_{n+i} at $C_i = 0$ is equal to the experimentally determined values of χ_n.

The equivalent conductance Λ_i of the ith salt in the presence of the other two electrolytes is obtained using the relation :

$$\Lambda_i = \frac{1000\ \chi_i}{C_i}$$

The values of Λ_i° were estimated from values at lower concentration by extrapolating the plots of Λ_i versus $\sqrt{C_i}$ to $\sqrt{C_i} \to 0$. The limiting equivalent conductance of the ith electrolyte in the systems A, B or C is in the order : KCl > NaCl > LiCl at all temperatures, despite the fact that the ionic crystal radius of Li^+ is the smallest and that of K^+, the largest. This reversed trend is caused by the solvent molecules tightly held by the intense electric field of the small ion[58]. On comparing these values with the individual values of the ith electrolyte, a greater increase in the limiting equivalent conductance has been recorded in the case of LiCl at 30°C. In general, the increase in the molality of the cations is in the order: Li > Na > K, at 30°C; the order is reversed at 50°C. Since the values of Λ_i° obtained are different from those given in the literature[4], it is clear that the conductance of the mixture is not additive to the contribution of the electrolytes.

Transport Numbers

The transport numbers can also be used for verifying the interionic attraction theory, but most of the existing data are of insufficient accuracy for this purpose. The study of transference numbers is an indirect method for the study of ion-ion and ion-solvent interactions. Single ion conductance values are useful for the investigation of ion-solvent interaction and they are related to the transference numbers as :

$$\lambda_i^\circ = t_i\, \Lambda_0 \qquad \ldots(40)$$

Experimental results have shown that transport numbers are generally concentration dependent and these findings are completely and quantitatively explained by the interionic attraction theory[59]. According to Onsager's limiting equation, the transport numbers vary linearly with $\sqrt{C}$. This is true for 1 : 1 electrolytes, but fails for polyvalent electrolytes and no equation has been proposed for a solution containing more than one electrolyte.

In a study of the equilibria existing between mixed salt solutions, Smith and Ball[60] and Smith and Braley[61]

noted that with equivalent mixtures NaCl and KCl in solution, the ion fraction of K gradually decreases with increasing total salt concentration. Braley and Hall[62] compared the calculated values (on the basis of isohydric principles) with the determined values of the transference numbers at various equivalent salt concentrations. The basis of comparison of the values obtained with the theoretical values was the ratio T_{Na}/T_K as obtained from Eq. (41).

$$\frac{T_{Na}}{T_K} = K \frac{\Lambda_{NaCl}/\Lambda_{0NaCl}\ \Lambda_{Na^+}}{\Lambda_{KCl}/\Lambda_{0KCl}\ \Lambda_{K^+}} \qquad \ldots(41)$$

No values were given for the cathode portion, as their results were very inconsistent. The calculated and observed ratios were plotted against the total concentration and it was observed that the theoretical ratio was always higher than the experimental ratio and the difference decreases as a function of the total concentration; this was explained on the basis of complex formation.

Schneider and Braley[63] carried out an extensive study on the composition of salt solutions and their degrees of association and dissociation. From a study of the transference number in a solution of NaCl and KCl it was found out that the solutions being dealt with were very complex. From the data, no definite conclusions could be drawn, but it was clear that the complex ions formed were of such a nature that their average mobility has no effect on the resistance of the solution. It was also noted that T_{Na}/T_K did not vary in direct proportion to the variation in the concentration ratio. The deviation decreased with increase in the proportion of KCl in the mixture. The possibility of complex formation due to hydration was also considered; it could not explain the data. The idea of complex formation was later contradicted by Barley and Rippie[64] who repeated the work with NaCl-KCl mixture. They showed that the transference numbers are additive and that the discrepancies (due to which the theory of complex formation was given) shown earlier were due to faults in the experimental method. Their work was further supported by MacInnes[65]. The closely linear variation of the conductance of the mixture, which was assumed in deriving Eq. (42) for transport numbers in a mixture, appeared to be good evidence for the presence of simple ions in the mixtures.

$$T_K = \frac{N_K\,(1-N_{Na})}{(N_K - N_{Na}) + (1-N_K)/x} \qquad \ldots(42)$$

Further evidence for the presence of simple ions was given by Dewey[66] and MacInnes *et al.*[67]. They studied NaCl-KCl mixtures employing different methods.

From the above it may be concluded that when the total concentration is not too high, the transport numbers of the two metallic ions are in satisfactory agreement with those calculated using MacInnes relationship[65] (43)

$$T_A = \frac{T_A^\circ\,(1-T_B^\circ)}{T_A^\circ - T_B^\circ + \dfrac{1-T_A^\circ}{x}} \qquad \ldots(43)$$

It follows that only simple ions are present in these solutions and that at equal concentrations, KCl and NaCl are dissociated to the same extent. This interpretation was discussed by Rysselberghe[68]. In a mixture of ACl and BCl the transport number was given by

$$T_A = \frac{x.\ \Lambda_{ACl}\,T_A^\circ}{x\Lambda_{ACl} + (1-x)\ \Lambda_{BCl}} \qquad \ldots(44)$$

This is a mathematical expression of the so-called isohydric principle and was derived on the assumption that the degree of dissociation and the mobilities of the ions of two salts in the mixture were the same as those in pure solutions of each of the two salts at the same concentration as the total concentration of the mixture. According to Rysselberghe, at concentrations not exceeding $0{\cdot}1N$, the alkali chlorides may be taken to be dissociated to the same extent at equal concentration. Since the direct calculation of mobilities in concentrated mixtures was probably impossible theoretically, interpretation of the experimental data was tried on the basis of the following hypothesis. In any mixture of two alkali chlorides the mobilities of the various ions are proportional to their values in solutions of the two salts of the same concentration as the total concentration of the mixture. The transport number in the mixture was given by

$$T_A = \frac{\alpha x\ T_A^\circ\ /T_{Cl,A}^\circ}{\alpha x[1+(T_A^\circ/T_{Cl,A}^\circ)+\beta(1-x)[1+(T_B^\circ/T_{Cl,A}^\circ)]} \qquad \ldots(45)$$

where α and β are the degrees of dissociation of ACl and BCl in the mixture. If $\alpha = \beta$, this relation is reduced to MacInnes formula[65]. These conclusions are in accordance with the findings of McBain and Rysselberghe[69] and Rysselberghe and Nutting[70] for RbCl-NaCl, CsCl–NaCl and KCl–LiCl mixtures.

No recent work has been reported on transport numbers in multicomponent electrolyte systems. The observations of different workers are contradictory and, therefore, it is necessary to carry out investigations with greater precision to explain the anomalies pointed out by different workers.

Viscous Flow

In a very dilute solution, the interstitial solvent between the cospheres of ions is unmodified and has the same properties as the pure solvent[71]. Each ionic species would be expected to contribute towards a change in the viscosity, but electrostatic forces between oppositely charged ions must be taken into account. The concentration dependence of electrolytic solutions has been interpreted in terms of semi-empirical Jones-Dole[72] equation :

$$\eta/\eta_0 = 1 + AC^{1/2} + BC \qquad ...(46)$$

where A is obtained from interionic theory[73,74] and B is an adjustable parameter, positive or negative, that accounts for ion-solvent interaction.

The solvents in which B values are negative, such as water, sulphuric acid, glycerol and ethylene glycol, have molecules capable of elaborating hydrogen bonds in three dimensions. The corresponding negative B values are found for monoatomic ions and for ions of low surface charge density.

It is believed[75] that the balance between the electrostatic effect in orienting the first solvation shell around the ion and the tendency exhibited by the molecules to stay as a part of the three-dimensional structure may lead to a structural collapse in that region and this makes B more negative. Such a disordered region may exist at the periphery of the region of dielectric saturation even for ions that are structure-makers and the B value of a particular ion may be seen as a result of positive and negative contributions[76]. The shape and size of electrolytes were correlated with the B coefficient through Vand's equation[77]. Stokes assumption of a rigid solvated volume, independent of concentration, is valid at high dilutions only. Einstein's equation[78] proposed a correlation between partial molal volume and viscosity. For small ions, the viscosity data indicated larger radii than did the molal volumes, as expected from electrostriction considerations; the agreement was quantitative for large ions.

So far, no theoretical and emprical study of the viscosity of multicomponent electrolyte solutions has been reported. Since viscosity β coefficients and the temperature dependence of β coefficient give a fairly good idea about ion-solvent interaction, it is necessary to study viscous flows in multicomponent electrolyte solutions, and to compare the conclusions drawn from these studies with those derived from thermodynamic properties and transport properties reported earlier.

The combined effect of salts can be studied in a similar manner as for a single electrolyte solutions using Eq. (46). Many equations have been proposed to represent the viscosity of mixtures of two liquids, but without any adequate theoretical basis it was not possible to say which equation corresponded to ideal behaviour[79]. As early as 1906, support was obtained for the equation of Bingham[79] :

$$\phi = \chi_A \phi_A + \chi_B \phi_B \qquad ...(47)$$

where ϕ is the fluidity of the mixture, and ϕ_A and ϕ_B are the values for the two pure components whose mole fractions in the mixture are χ_A and χ_B respectively. A more satisfactory theoretical relationship was later given by Kendall

$$\log \phi = \chi_A \log \phi_A + \chi_B \log \phi_B \qquad ...(48)$$

Banchotti[80] carried out viscosity measurements on an aqueous mixture of K_2SO_4 and $ZnSO_4$ in an attempt to detect the formation of double salts. With $0{\cdot}1M$ solutions, the viscosity values agreed well with the values calculated from the law of mixtures but with $0{\cdot}5M$ solutions, the viscosities were higher than the values calculated from the law of mixtures. The differences between the experimental and calculated values were greatest with equimolar proportions of the salts. This indicates the formation of an ionic complex or a molecular addition compound between the two salts. Hitchcock and McIlkenny[81] also showed that in $NaOH$-Na_2CO_3 and KOH–K_2CO_3 mixtures up to concentration $8N$, the viscosities deviated from the mixture rule. Tollert[82] made viscosity measurements on 8 mixtures of aqueous solutions of strong electrolytes at total concentrations of $0{\cdot}1N$ and $1{\cdot}0N$. Eq. (49) has been found to represent the viscosity of mixed unreacting salt solutions[83].

$$\eta = \frac{\eta_1}{1 + K[Z_m/(1-Z)]} + \frac{\eta_2}{1+(1/K)[(1-Z_m)/Z_m]} \qquad ...(49)$$

where, η_1, η_2, and η are viscosities of components 1,2 and the mixture respectively; K is a characteristic constant for the mixture; and Z_m is the mole fraction of component in the mixture.

$CuSO_4$–KCl and $CuSO_4$–H_2SO_4 mixtures were studied by Asmus[84] as a function of molecular concentration and the total ionic concentration. At high dilution, the relative viscosity of a mixture of the strong electrolytes obeyed the relation :

$$\eta/\eta_o = 1 + a\sqrt{\Omega} + b\Omega \qquad ...(50)$$

where a and b are empirical constants, and Ω the total ionic concentration.

The viscosities of mixed solutions of NaCl, KCl and NH_4Cl were measured by Zdanovskii[85]. Later, Onsager and Fuoss's limiting law for mixed ionic solutions was verified by Chakravarti and Prasad[86]. They obtained a straight line for mixed solutions of $BaCl_2$ + NaCl and $MgCl_2$ + NaCl by plotting $(\eta/\eta_0 - 1)\sqrt{C}$ against $\sqrt{C}$. The coefficient A in $\eta/\eta_0 = 1 + A\sqrt{C} + B\,C$ was shown to be a linear function of composition in the low concentration range[87]. The relative viscosities η/η_0 for the mixtures[88] K_2SO_4–KCl, $NaNO_3$–NaCl, $NaNO_3$–KCl and $NaNO_3$–HCl can be represented by Jones-Dole equation and the coefficients A and B were linear functions of $C_1/(C_1+C_2)$. Grunberg and Mission[89] showed that the expression $\log \eta_s = N_1 \log \eta_1 + N_2 \log \eta_2 + N_1 N_2 d$ fits the data more closely than the following Arrhenius equation[90]:

$$\log \eta_s = N_1 \log \eta_1 + N_2 \log \eta_2 \qquad ...(51)$$

Tollert[91] showed that the dynamic viscosities of aqueous solutions of pairs of electrolytes could be calculated by the sum rule.

Diffusion

In a ternary system of two solutes and one solvent, there exist two diffusional flows that may exhibit coupling phenomenon. Coupling of two transport processes can be best explained with the help of the theory of thermodynamics of irreversible processes[92,93], two straight coefficients, D_{11} and D_{22}, and two cross-coefficients, D_{12} and D_{21}. These diffusion coefficients are given by :

$$D_{11} = L_{11}\mu_{11} + L_{12}\mu_{21} \; ; \; D_{12} = L_{11}\mu_{12} + L_{12}\mu_{22}$$
$$D_{21} = L_{21}\mu_{11} + L_{22}\mu_{21} \; ; \; D_{22} = L_{21}\mu_{12} + L_{22}\mu_{22} \quad \ldots(52)$$

where $\mu_{kj}(x,j = 1,2)$ are chemical potential derivatives

$$\mu_{xj} = \frac{\delta\mu_x}{\delta C_j} \quad \ldots(53)$$

It is observed that the thermodynamic requirement $L_{12} = L_{21}$ does not lead to equality of the coefficients D_{12} and D_{21}, nor does the vanishing of the hydrodynamic coupling, $L_{12}=0$ lead to $D_{12}=0$. When both hydrodynamic and thermodynamic coupling vanish, i.e. $\mu_{12}=\mu_{21}=0$ and $L_{12}=0$, the cross-diffusion coefficients vanish. Dunlop and Gosting[94] first measured diffusion coefficients for a three-component system. The diffusion process has since been studied for several non-electrolyte and electrolyte ternary systems. Especially because of the many multicomponent electrolyte systems of industrial and biological interest, it is important to estimate the phenomenological coefficients, $(L_{ij})_0$, and diffusion coefficients, $(D_{ij})_0$, for such systems. The coefficients actually measured in diffusion experiments refer to cell fixed frame of reference, $(D_{ij})_0$, and these are very nearly equal[95] to volume fixed diffusion coefficients $(D_{ij})_v$; the $(D_{ij})_0$, are related to the latter coefficients[96] by :

$$(D_{ij})_0 = (D_{ij})_v + \frac{C_i}{C_0 V_0} \sum_{k=1}^{n-1} \bar{V}_k (D_{xj})_v \ldots (ij = 1 \ldots n\text{-}1) \quad \ldots(54)$$

where V_k is the partial molar volume of the component k. It is seen that near infinite dilution $(D_{ij})_0 = (D_{ij})_v$, but even in relatively concentrated solutions, $(D_{ij})_0$ and $(D_{ij})_v$ may differ by only 1–5%. Dunlop and Gosting[94,97] showed that Fick's law is generally not valid for systems of three or more components. Therefore, it is important to recognize that the use of functions derived from Fick's first law to calculate a single diffusion coefficients for each solute in a binary or ternary system is only an approximate procedure[98–101] for representing the diffusion process. Both Lamm[102] and Onsager[103] proposed sets of generalized flow equations to describe flows in systems of three or more components. Baldwin *et al.*[104] adopted the Onsager Eq. (55) as a direct phenomenological approach :

$$J_1 = -D_{11}\left(\frac{\delta C_1}{\delta x}\right)_t - D_{12}\left(\frac{\delta C_2}{\delta x}\right)_t$$
$$J_2 = -D_{21}\left(\frac{\delta C_1}{\delta x}\right)_t - D_{22}\left(\frac{\delta C_2}{\delta x}\right)_t \quad \ldots(55)$$

The four diffusion coefficients used to describe the systems LiCl–NaCl and LiCl—KCl were determined by using the Gouy diffusionmeter. For determining the diffusion coefficients, these workers adopted a general method of interpreting refractive index gradient curves. The applicability of this method was limited because of the inaccuracy involved in interpreting the curves, and Dunlop and Gosting[94] adopted a slightly different procedure to calculate the values of diffusion coefficients. This method was based on series expansions for the concentration curves which are applicable only when one cross-term diffusion coefficients is very small. To overcome the limitations of the above two methods, Fujita and Gosting[105] used exact solutions derived for the solute concentration distribution in free diffusion, to devise new procedures for calculating the four diffusion coefficients. From these experiments, it was concluded that three-component systems consisting of an electrolyte and a non-electrolyte in water or two non-electrolytes in water would not exhibit measurable flow interactions and Fick's law could be used. Dunlop[106] reported diffusion data for the system raffinose-KCl–H_2O and showed that measurable interaction of flows did exist, indicating the use of generalized flow Eq. (55). In view of the increasing interest in experimental studies of the interaction of flows in multicomponent systems, Fujita[107] developed an additional approach for accurate evaluation of both main and cross-term diffusion coefficients.

Dunlop and Gosting[2] made tests of the Onsager's reciprocity relations for ternary diffusion in relatively dilute (total concentration less than 1*M*) solutions of the system NaCl-KCl-H_2O. Dunlop[108] repeated the same work with two objectives : it was hoped that (i) at high concentrations the cross-term diffusion coefficients would be larger than those in the previous experiments; and (ii) at high concentrations the derivatives of the logarithms of the activity coefficients contribute a greater proportion of the chemical potential derivatives, which appear in the Onsager relation for a ternary system than they do at lower concentrations. No conclusion could be arrived at because the concentrations used by Dunlop for these chemical potential derivatives are not as accurately known as they are at lower concentrations.

A procedure developed by Fujita and Gosting[109] is claimed to permit calculation of the four diffusion coefficients at a given composition of a ternary system from suitable free diffusion experiments performed with the Gouy diffusionmeter. The new procedure could be applied regardless of the magnitudes of the diffusion coefficients. The data required were the reduced height-area ratios of the refractive index gradient curves and the areas of graphs of Guoy fringe deviations which summarize deviations of the refractive index gradient curves from Gaussian shape. Data for the system NaCl-KCl-H_2O reported previously were reanalysed to illustrate the use of the procedure. It was found that the new values of the diffusion coefficients so obtained satisfied the ORR better than did the values calculated earlier. All these tests for the ORR for ternary isothermal diffusion were, except for one case, subject to the limitation that experimental data for the diffusion coefficients and activity coefficient had not been determined in the same composition range. Woolf *et al.*[110] used various procedures to approximate the activity coefficients in the concentration range where diffusion had to be studied. They verified the ORR for H_2O-glycine-KCl system by measuring the diffusion coefficients using Gouy interference method.

Wendt[111] reported values of diffusion coefficient measured with Guoy diffusionmeter for four compositions of the system H_2O-Na_2SO_4-H_2SO_4. This system was particularly interesting, because the solute H_2SO_4 was expected to exhibit the properties of both a strong acid (H_2SO_4) and a weak acid (HSO_4^-). Relatively large values were expected for the cross-term diffusion coefficients, $(D_{12})_v$ and $(D_{21})_v$, because of the appreciable concentrations of very mobile H^+ in the solutions The measured values of these coefficients for some compositions were found to be larger than the values previously reported for ternary systems. The values of the diffusion coefficients $(D_{12})_v$ and $(D_{21})_v$ were found to be generally larger, from which it may be inferred that in any ternary system of electrolytes containing a strong acid as one solute, or, in general, any multicomponent system of electrolytes containing at least two ions of considerably different mobilities, would be expected to have a large value for at least one cross-term coefficient.

Burchard and Toon[112] first made studies on a very interesting class of multicomponent systems made up of completely miscible non-electrolyte liquids. The object of this study was to determine the rates of diffusion in a ternary system as functions of the concentrations and concentration gradients over the complete range of compositions. A modification of the diaphragm cell was used to investigate isothermal diffusion in the thermodynamically near non-ideal liquid system, toluene-chlorobenzene-bromobenzene. It was shown from the phenomenological equations that in the absence of cross-term diffusion coefficients, the main coefficients were equal. This was approximately true for the system studied, for the cross-diffusion coefficients and the differences between the main diffusion coefficients were of an order of magnitude less than the main diffusion coefficients.

A knowledge of the diffusion coefficients in three-component solutions containing in addition to sucrose either electrolytes, such as KCl, or other sugars, is of value in understanding the phenomena of growth of sugar crystals. For this reason, Hension[113] studied the systems sucrose-KCl-H_2O, sucrose-glucose-H_2O and sucrose-fructose-H_2O using the diaphragm cell method. It was observed that at a given sucrose concentration, there was very little difference between the values of the diffusion coefficients (D_{11}) for sucrose in H_2O and sucrose in H_2O and in 1*M* KCl solution. On the other hand, the value of D_{22}, which describes the diffusion of KCl in the presence of uniform concentration of sucrose, was strongly affected by the presence of sucrose. The approximate constancy of D_{11}/D_{12} at different sucrose concentrations meant that in transporting KCl, a given molar concentration gradient of sucrose is about 30% as effective as the same molar concentration gradient of KCl.

Wendt[114] derived equations valid at low concentrations for estimating phenomenological coefficients and diffusion coefficients of ternary systems. The four phenomenological coefficients $(L_{ij})_o$ for a ternary system are estimated from the values of the limiting equivalent conductivities and the concentrations of the ions. From estimates of $(L_{ij})_o$ and from either known or calculated values of the chemical potential derivatives, the four diffusion coefficients for the system could be calculated using the equation :

$$(D_{ij})_o = \sum_{k=1}^{n-1} (L_{ik})_o \, \mu_{kj} \; (i,j = 1 \ldots . n-1) \qquad \ldots(56)$$

where n is the number of components. Estimated values were compared with the experimental values for the system H_2O -NaCl-KCl, H_2O-LiCl-KCl, H_2O-LiCl-NaCl and H_2O-Na_2SO_4-H_2SO_4. For the three strong electrolyte systems, the predicted and observed values of $(D_{ij})_o$ were found to agree remarkably well. However, for the weak electrolyte system H_2O -Na_2SO_4-H_2SO_4, the agreement was relatively poor.

Miller[115] verified the ORR for H_2O-NaCl-KCl system using the activity data over the whole range of concentration supplied by Robinson[116] and the values of $(D_{ij})_o$ supplied by Fujita and Gosting[109]. The procedure of Fujita and Gosting[109] was extended to 4-component systems by Kim[117]. It was also predicted that for a system with $n+1$ components, n different experimental quantities have to be obtained from n different experiments at given mean solute concentrations in order to evaluate n^2 diffusion coefficients. However, many difficulties are encountered when the number of components is increased. The most successful theory for predicting diffusion coefficients for ternary systems was derived by Miller. He combined a rigorous application of irreversible thermodynamics with methods of estimating ternary transport coefficients and activity coefficients, derivatives from binary data. To test this theory, Wendt and Shamim[118] calculated the diffusion coefficients experimentally for the system $MgCl_2$-NaCl-H_2O. Unfortunately, transference and diffusion data for the system $MgCl_2$-H_2O are not available, hence, the diffusion coefficients could not be estimated by Miller's methodology. The large cross-term diffusion coefficients D_{12} and D_{21} measured for each of the compositions studied in this work emphasize that the electrolyte solutes in multicomponent systems diffuse independently.

Recently, Kim *et al.*[119] studied H_2O-KCl-HCl and H_2O-NaCl-HCl systems over a wide composition range to elucidate the general diffusion behaviour. $(D_{12})_v$ was found to have a large negative size. This was due to the fact that the mobility of the proton is much greater than that of the chloride ion and the resulting large electrical potential gradient induces the potassium ion to move against its own concentration gradient. Also, the value of $(D_{12})_v$ was larger for H_2O-KCl-HCl system than for H_2O-NaCl-HCl system because of the greater mobility of K^+. In H_2O-HCl system, the faster proton ion is slowed by the slower chloride ion to maintain the macroscopic electroneutrality condition. In the presence of an additional electrolyte, NaCl or KCl, the electroneutrality conditions imposes less restraint on the proton and thus $(D_{22})_v$ may have larger values than the binary diffusion coefficient. In general, it was found that the magnitudes of the cross-term diffusion coefficients, D_{ij}, depend on three factors, viz. (i) con-

centration of the component i; (ii) mobility of the component i; and (iii) the force produced by a unit concentration gradient of the component j.

Wendt's methodology for ternary systems was applied to biofluids[120]. The biofluids studiedcontain thesame constituents[19], viz. NaCl, KCl, $CaCl_2$ and $NaHCO_3$ atdifferent concentration. The estimations showed that the ORR is valid in biofluids. Estimation of the diffusion coefficients for various constituents of biofluids helps in interpreting the preferential diffusional loss of the constituents as neutral salts or ions. For the two biofluids studied, the rate of loss of sodium chloride in the case of amphibians is less in comparison to fresh water fish, and, therefore, to balance the concentration, sodium pump mechanism, will be favoured in the case of fresh water fish and not amphibians.

Phenomenological and diffusion coefficients have also been calculated for LiCl-NaCl-KCl-H_2O and LiCl-NaCl-KCl-DMSO systems. Parallel behaviour is observed in the two systems, and the ORR is found to be valid in both cases. However, the magnitude of the diffusion coefficients of the solutes is found to be comparatively much smaller in DMSO, at the same concentration as in water. This has been attributed to the lesser mobility of the ions in DMSO. It is also observed that in these multicomponent systems, K^+ has the greatest mobility and the mobilities of the ions are in the order: $K^+ > Na^+ > Li^+$. From the comparison of diffusion coefficients of electrolytes in these multicomponent systems with those in the binary systems, it is found that the mobilities of the ions are considerably suppressed in the multicomponent systems.

Summary

Recent advances in respect of the thermodynamic properties, such as activity coefficient, free energy change, entropy change, partial molar properties of systems involving mixed electrolytes, and transport properties, such as electrical conductance, viscous flow, transference numbers and diffusion of multicomponent electrolyte systems have been reviewed.

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Apoproteins of Human Plasma Lipoproteins

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THE lipoproteins transport lipids in the plasma and are composed mainly of lipids and proteins. Small amounts of carbohydrates are also present. The structure of lipoproteins is determined by the relative amounts of various lipids and proteins and has a profound influence on their physical and chemical properties, metabolism and physiological functions[1–3]. In the plasma, these macromolecules are present as several discrete species of particles that vary in hydrated density, size and electrophoretic mobility. They also vary in their contents of protein, carbohydrates, triacylglycerol, unesterified cholesterol, cholesterol esters and phospholipids. In normal subjects, the concentration and distribution of the various lipoprotein species fall within fairly well defined ranges. Abnormal concentrations of plasma lipoproteins with or without change in their composition are usually associated with various physiological and pathological conditions. One of the approaches to study structure-function relationships of the lipoproteins has been to analyze the lipid and protein moieties in various physiological and pathological states. In this regard, until recently, attention was focused mostly on the lipid components. Extensive knowledge of triacylglycerol and cholesterol metabolism has been gained, but very little is known about the origin, synthesis and ultimate fate of the apoproteins[1–3]. Due to the development of procedures for obtaining lipid-free apoproteins, and advances in the techniques of protein chemistry, many apoproteins have been isolated and characterized in the last decade[4]. This has led to some understanding of the role of the apoproteins in lipoprotein structure and metabolism. The purpose of this review is to summarize current knowledge about the structure and composition of protein moiety of the various lipoproteins and to indicate current trends in lipoprotein research.

Isolation and Characterization of Lipoproteins

The classification of the lipoproteins into 4 major families is based on operational criteria like hydrated density and electrophoretic mobility. Based on their density and behaviour in the ultracentrifuge, they have been described as chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). Each of these classes can be further fractionated into subclasses on the basis of their density. According to their electrophoretic migration on paper, agarose or cellulose, the four families of lipoproteins have also been designated as α (HDL), β(LDL), pre-β (VLDL) and chylomicrons. The chylomicrons remain at the origin on electrophoresis. The hydrated density of the lipoproteins is primarily a consequence of their lipid content, whereas electrophoretic mobility is determined principally by their protein moieties. Recently, Alaupovic *et al.*[5,6] have proposed a concept for classification of lipoproteins based on apoproteins. According to this scheme, there are three major lipoprotein families, namely LP-A, LP-B and LP-C, containing apoprotein A, apoprotein B and apoprotein C, respectively, as their specific proteins (*see* apoprotein nomenclature for details). Very recently, one more distinct family called LP-D having apoprotein D as its specific apoprotein has been recognized[7]. The general properties and lipid composition of human plasma lipoproteins are summarized in Table 1[1,2,8]. Many excellent articles can be recommended for more information on lipoproteins: structure and metabolism[1–3,8–12], interactions with polyanions and metals[13] and electron microscopy[8].

Nomenclature, Isolation and Estimation of Apoproteins

Nomenclature — The protein component of lipoproteins has been fractionated into several constituent apoproteins[4–7,14] differing in immunological properties, sedimentation coefficient, molecular weight, amino acid composition, amino and carboxyl terminal amino acids and carbohydrate content (Table 2)[4–7,14]. A standard nomenclature for apoproteins is not available yet. Therefore, operational criteria based on the carboxyl-terminal amino acid of the polypeptide[14,17–21] order of elution from Sephadex columns[12,22], or A, B, C nomenclature proposed by Alaupovic *et al.*[5,6] are used. At present, A,B,C nomenclature seems to be popular, mainly because of its simplicity. In this system, the major apoprotein components of HDL, LDL and VLDL are apo A, apo B and apo C, respectively. Each group of apoproteins is heterogeneous, containing more than one polypeptide chain. For example, apo A contains AI and AII (Table 3). Moreover, the VLDL, LDL and HDL fractions isolated by ultracentrifugation contain all these apoproteins in different proportions (Table 3). The presence of an apoprotein in a particular density class is probably a function of its lipid binding capacity and, to some extent, reflects the protein and lipid exchange processes between lipo protein families.

Delipidation — To obtain lipid-free apoproteins the isolated lipoprotein fractions are usually delipidated by extraction with ethanol-diethyl ether[17,23–29], chloroform-methanol[28] or 2-butanol-acetic acid-water mixtures[30]. The delipidation procedures for VLDL, LDL and HDL, recommended by Scanu and coworkers[24,28], are listed in Table 4. Among the problems faced during delipidation are: (1) incomplete recovery of apoproteins due to loss of some polypeptides in the organic solvents, and (2) formation of water-insoluble aggregates, particularly with apoprotein B. The losses in the organic solvents can be minimized by using a proper ratio of ethanol-diethyl ether[28]. Several procedures have been described to obtain totally water-soluble apoproteins. Some of these procedures have used chemical modifications (acetylation[24], succinylation[24,29,31,32] maleylation[25]), denaturants (urea[23,26], guanidine hydrochloride[25,33]), high *p*H solutions[29,32] and detergents (sodium deoxycholate[34,35], Nonidet P-40[35], sodium dodecyl sulphate[24,35] and cetyltrimethyl ammonium bromide[35]).

TABLE 1 — GENERAL PHYSICOCHEMICAL PROPERTIES OF HUMAN SERUM LIPOPROTEINS

	Chylomicrons	VLDL	LDL_1	LDL_2	HDL_2	HDL_3
Solvent density, g/ml	<0·95	0·95–1·006	1·006–1·019	1·019–1·063	1·063–1·125	1·125–1·21
Flotation rate S_f (1·063)[a]	>400	20–400	12–20	0–12	—	—
S_f (1·21)[b]	—	—	—	—	3.5–9	0–3·5
Hydrated density, g/ml	0·93	0·93–1·01	1·003	1·030	1·08–1·12	1·12–1·16
Diameter, nm	75–600	30–90	22–24	20–22	6–14	4–10
Molecular weight	$>0{\cdot}4\times10^9$	$5–10\times10^6$	$4–5\times10^6$	$2{\cdot}75\times10^6$	$3{\cdot}6\times10^5$	$1{\cdot}51\times10^5$
Electrophoretic mobility						
Paper, agarose	origin	Pre-β	—	β	α	α
Starch block	α_2,β	α_2	—	β_1	α_1	α_1
Av. Composition, % by wt						
				(LDL_1+LDL_2)		(HDL_2+HDL_3)
Triacylglycerols	84	51		11		4
Cholesterol (free)	2	7		8		2
Cholesterol esters	5	13		37		20
Phospholipids	7	19		22		24
Protein	2	8		21		50

a-S_f denotes flotation rates expressed as Svedbergs (10^{-13} cm/sec/dyne/g) in a sodium chloride solution of density 1·063 g/ml at 26°C.
b- Analogous to S_f measured at solution density 1·210 g/ml

Helenius and Simons[35] have described a procedure by which water-soluble apo LDL can be obtained without the use of organic solvents. In this procedure, the lipoprotein is mixed with the detergent, and the protein is separated from lipid-detergent micelles by gel filtration in the presence of the detergent. The detergent is removed from the protein by gel filtration in a solvent containing no detergent. Using this procedure, sodium deoxycholate and Nonidet P-40 did not change the immunological properties of Apo LDL. By contrast, sodium dodecyl sulphate and cetyltrimethyl bromide did produce a change in immunological properties.

An ideal delipidation procedure should yield a product completely free of lipids and soluble in aqueous medium. In addition, the product should retain its native chemical, physical and immunological properties and its capacity to combine with lipids. None of the current methods meets all these requirements, because after delipidation, a small amount of phospholipids (1-3% by weight) always remains with the apoprotein[10,11], and the conformation of apoprotein is altered[10]. Moreover, although apo A and apo C polypeptides can be dissolved in aqueous buffers of appropriate *p*H and ionic strength, the apo B peptides cannot be solubilized without some dissociating agents[10-12,24].

Apoprotein isolation and estimation — The lipid-free apoproteins can be fractionated and isolated in pure form by gel filtration (sephadex[12,17] and sepharose[36]), ionexchange chromatography[17,26,27,37] and isoelectric focussing[38-41]. The apoproteins can also be separated and identified by polyacrylamide gel electrophoresis in 8 *M* urea[27,37] or in 0·1 *M* sodium dodecyl sulphate[43]. To ensure dissociation of the various apoproteins during column chromatography and electrophoresis, dissociating agents like urea[27,37,43] guanidine[36] or sodium dodecyl sulphate[42] are usually added to the buffers.

These methods are invaluable for preparative work and to isolate individual apoproteins in pure form. However, they have limited value for quantitation of apoproteins in the intact plasma and tissues. The apoproteins are now viewed as important determinants of structure and porperties of lipoproteins. They also act as modulators of interactions among various lipoprotein species and with other tissues, and their quantitative patterns can provide useful information in understanding the mechanisms underlying lipid disorders. Therefore, there is a need for simple and accurate methods for the quantitative estimation of different apoproteins in the plasma of patients with various diseases. Moreover, the quantitative estimation of minute amounts of apoproteins and their localization in intestine and liver should increase our knowledge about the synthesis of apoproteins, their assembly into lipoproteins, the secretion of lipoproteins and the ultimate fate of apoproteins in the body.

At present, immunological methods are most promising for quantitative estimation of the apoproteins. These methods have the advantage of being simple, precise, specific, accurate and sensitive for apoprotein estimation. Specific antibodies for human apoproteins AI, AII, B, C, arginine-rich and D are already available, and radioimmunoassays for these proteins are being developed[6,7,10]. Radioimmunoassays for human apoproteins AI[44-47] and B[48-51] and rat apoproteins AI[52] and B[53] have been reported. In these procedures, the plasma or tissue extracts have to be delipidated or heated at 50°C for 3 hr to expose all the reactive

TABLE 2 — POLYPEPTIDES OF HUMAN SERUM LIPOPROTEINS[a,b]

Amino acids	A-I	A-II	A-III[c]	D[d]	D_2[e]	B	Arg-rich protein	C-I	C-II	C-III-0, C-III-1[f] and C-III-2[g]
Asp	75	34	79	83	48	95	51	81	64	80
Thr	36	69	43	45	65	58	34	45	101	57
Ser	50	69	84	36	76	76	45	106	99	125
Pro	36	46	34	70	55	29	13	15	26	23
Glu	168	184	137	100	201	102	135	136	104	114
Gly	36	34	71	34	50	39	51	15	28	34
Ala	68	57	47	51	68	54	78	45	75	114
Cys $\frac{1}{2}$	0	11	0	10	17	3	0	0	0	0
Val	46	69	51	50	71	43	55	30	45	68
Met	11	11	9	6	10	12	5	15	21	23
Ile	0–4	11	17	37	14	46	13	45	10	0
Leu	139	92	64	56	104	98	90	91	92	57
Tyr	25	46	23	26	47	28	13	0	53	23
Phe	21	46	27	29	50	43	16	45	24	46
Lys	75	103	64	59	118	70	37	136	70	68
His	18	0	13	11	2	19	6	0	0	11
Arg	57	0	23	18	6	26	69	45	13	23
Trp	14	0	ND[h]	3	13	13	24	15	—	34
Carbohydrates, % by wt	0	0	ND	+	0	3–4	ND	0	0	2–3
NH_2-terminus	Asp	PCA[i]	ND	ND	Leu	Glu?	Lys	Thr	Thr	Ser
COOH-terminus	Gln	Gln	Ser	ND	Ser	Ser?	Ala	Ser	Glu	Ala
Mol. wt.	28,016	8707	20,000		7000	j	33,000	6620	10,000	8764[k]

[a]Table adapted from Ref. 10.
[b]Moles/100,000 g protein
[c]Data from Ref. 15 . The composition, although similar, differs significantly from that of apoD reported by McConathy and Alaupovic.
[d]According to McConathy and Alaupovic[7] apoD contains 29.2 moles glucosamine/100,000 g protein.
[e]Ref. 16.
[f]Contains 1 mole sialic acid/mole protein
[g]Contains 2 moles sialic acid/mole protein
[h]ND, not determined
[i]pyrrolidone carboxylic acid
[j]Not firmly established
[k]Calculated on amino acid composition only

sites for accurate estimations of the apoproteins. Besides these immunological methods, Kane[54] has reported a simple procedure for the estimation of apoprotein B in isolated lipoprotein fractions. Apoportein B is insoluble in 50% tetramethyl urea, whereas other apoproteins are soluble in this solvent. The tetramethyl urea-soluble apoproteins can be resolved on urea-polyacrylamide gels and estimated by densitometric scanning.

Apoprotein Composition of Lipoproteins

Chylomicrons — The protein content of chylomicrons is low and variable. The presence of apoprotein C (66%), apoprotein B (22%) and apoprotein A (15%) in human lymph chyle preparations has been reported [55,56]. Apoprotein AI and AII were found in 1:1 weight ratio in chylomicrons. By contrast their weight ratio is 3:1 in human HDL[12]. The larger chylomicrons have a greater proportion of apoprotein B than the smaller ones[57]. Moreover, these chylomicrons lose apoprotein B and gain apoprotein C when incubated with serum[57].

VLDL — The protein moiety of VLDL contains several distinct apoproteins. The major ones belong to the apoprotein C (40-80%) and apoprotein B (about 40%) families[10,12,58]. The proportion of the various apoproteins varies with the individual and the degree of hyperlipidemia[27,59]. The apoprotein composition of VLDL differs from one subfraction to another[27,60]. The larger the VLDL particle, the greater is the amount of apoprotein C relative to apoprotein B[60]. In normal VLDL, the arginine-rich

apoprotein content approximately equals that of apoprotein C II, but it is about one-third of the C III peptides[61]. Apoprotein A is usually absent in normal VLDL, but trace amounts (<1%) can be demonstrated by immunochemical means[54,62,63].

LDL — Apoprotein B makes up 92-98% of the human LDL apoproteins[62,64]. Small quantities of apoproteins C and A have also been reported in various fractions of human LDL by Lee and Alaupovic[65,66].

Table 3 — Apoprotein Composition of Human Plasma Lipoproteins[a]

Apoprotein	Chylomicrons	VLDL	LDL	HDL
	(% of total lipoprotein protein)			
A I	?	T[b]	—	65–70
A II	?	?	—	20–25
A III or D	—	—	—	*T*
B				
CI	5–22?	40	>95	*T*?
C II				1–3
C III-0			*T*	1–3
C III-1	66	60		
C III-2				5–10
Arginine-rich (ARP)	—	*T*	—	*T*?

[a] For references see text on apoprotein composition of lipoproteins
[b] *T*, amounts less than 1%

The lower the density, the lesser is the proportion of apoprotein B in these particles. Kostner[67] has isolated a lipoprotein in the density range of HDL (*d*,1·073-1·125) from the plasma of healthy women that contains only apoprotein B. This lipoprotein is distinct from allotype Lp (a) lipoproteins and has been purified by immunoadsorption. It is referred to as LP-β_{HDL}.

HDL — The apoproteins of human HDL are composed of two major proteins (AI and AII) and many minor components[5,10,12,37]. About 90% of the protein of HDL can be accounted for by apoproteins AI and AII, and their ratio may vary from one subfraction of HDL to another[62,68]. The molar ratio of apoprotein AI/AII has been found to be greater in HDL_2 (9:1) than in HDL_3 (2:1), as determinedby immunochemical methods[62]. In contrast, by using disc gel electrophoresis of fluorescence-tagged apoproteins, Friedberg and Reynolds[69] have found a molar ratio of 2:1 between apoprotein AI and apoprotein AII in HDL_2, HDL_3 and total HDL. The differences in the values for HDL_2, reported by the two groups, might have been due to inaccessibility of the antigenic determinants of HDL_2 to the antibody in the immunochemical method used by Kostner *et al.*[62]. A weight ratio of 3:1 (AI:AII), which corresponds to molar ratio of 2:1 in human HDL_2 and HDL_3, also has been reported by Scanu *et al.*[22].

Apoprotein C[6,70], thin-line protein[15] and arginine-rich protein[70] have also been found in human HDL fraction in very small amounts. Recently, Lim *et al.*[16] have shown the presence of a small peptide, D_2, in plasma HDL of normal and abetalipoproteinemic subjects. This peptide has a molecular weight

Table 4 — Delipidation of Lipoproteins

General scheme

The lipoprotein solution (2-5 mg protein/ml)

↓ Ethanol:diethyl ether treatment(2-4 hr, –10°C, centrifugation)[a]

Precipitate

↓ Diethyl ether treatment (overnight, –10°C), centrifugation

Precipitate

↓ Wash 3 times with diethyl ether at 4°C and dry under a stream of nitrogen

Apo VLDL[(b)]: soluble in 0·1 *M* Tris (*p*H 8·6)
Apo LDL[(b)]: soluble in 0·1 *M* Tris (*p*H 8·6), 0·2 *M* SDS, 2-6 hr, 40°C
Apo HDL[(b)]: soluble in dilute aqueous buffers at *p*H 7·0

Comments

VLDL and HDL: 0·15 *M* NaCl, 10^{-3} *M* EDTA, *p*H 7.0
LDL: 0.15 *M* NaCl, 10^{-3} *M* EDTA, 0. 2*M* sodium dodecyl sulphate, *p*H 7.0, incubate at 40°C for 90 min before delipidation.
Ratio of ethanol:diethyl ether (V/V)
VLDL 3:1
LDL 1:3
HDL 3:2

[(a)]For complete protein recovery adjust ethanol-diethyl ether ratio of the first extract to 3:5; leave overnight at –10°C, collect precipitate by centrifugation and combine with apo VLDL or apo HDL.
[(b)]Apo VLDL, apo LDL and apo HDL refer to lipid-free protein moiety of VLDL, LDL and HDL, respectively.

of 7000, and its electrophoretic mobility, chemical and immunological properties are distinct from those of the known apoproteins C.

Properties of Apoproteins

Apoproteins AI and AII — Apoprotein AI, the major protein of human HDL, has a molecular weight of 28,000 daltons[4,42]. It is composed of a single polypeptide chain of 245 amino acid residues[71] (Fig. 1). AI contains 3 residues of methionine and 4 of tryptophan, but no cysteine, isoleucine or disulphide linkage. Apoprotein AI seems to be microchemically heterogeneous[42,72]. According to Edelstein *et al.*[42], it contains two major polymorphic components (IIIa and IIIb) having identical molecular weights and immunological properties, but with slight differences in amino acid composition and electrophoretic mobility.

Apoprotein AII is the second most abundant protein in HDL. Human AII has a molecular weight of 17,400[73]. It is composed of two identical polypeptide chains of 77 amino acids each, linked by a single disulphide bond at cystine-6 (Fig. 2)[20,74–78]. Its analogous protein in rhesus monkey plasma exists as a monomer with a molecular weight of 8750[79–81].

Apoprotein B — Very little is known about the physical and chemical properties, and composition of apoprotein B. Many attempts to characterize this protein have failed, because it forms water-insoluble aggregates in the lipid-free state[24]. For this reason, dissociating agents like sodium deoxycholate[34,35] and sodium dodecyl sulphate[24,64] or denaturants like urea[26] and guanidine[25,33] are included in the buffer before delipidating the LDL and VLDL fractions. Chemical modifications of LDL, like succinylation[31,32] and maleylation[25], and high *p*H solutions[29,32] have also been used. Depending on the method used for delipidation and isolation of the protein, estimates of the molecular weight of the apoprotein subunits vary from 8,000 to 275,000[4]. Subunit heterogeneity has also been suggested by immunochemical analysis[82], isolation of many components differing in amino acid composition by DEAE-cellulose columns[26] and the identification of two distinct fractions with different amino acid compositions after sephadex chromatography of the maleylated apoproteins[25]. The wide variations in the molecular weight values for apoprotein B subunits have been attributed to the presence of a protease-like activity in LDL[83]. Chen and Aladjem[34] have shown the absence of such activity in LDL. They obtained different molecular weight values when either organic solvents or sodium deoxycholate were used for delipidation. Delipidation by organic solvents induces irreversible aggregation of apo LDL. When LDL is delipidated with sodium deoxycholate, two fundamental subunits of molecular weight 9,500 and 13,000 can be identified by sodium dodecyl sulphate-polyacrylamide gel electrophoresis[34]. It has been suggested that these two subunits form oligomers to yield various components of higher molecular weights[34].

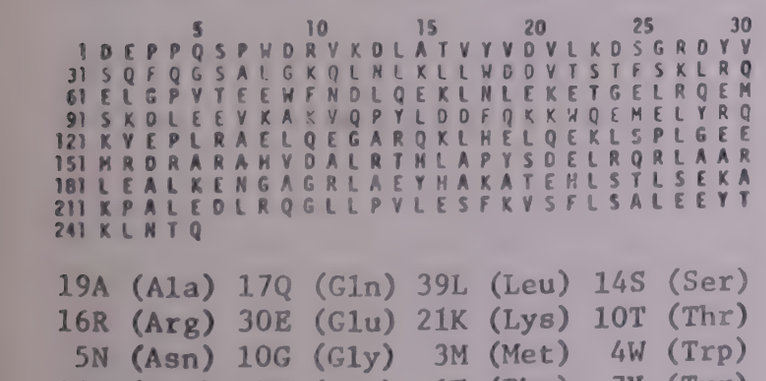

Fig. 1 — Amino acid sequence of human apoprotein AI using one letter notation for amino acids[71]

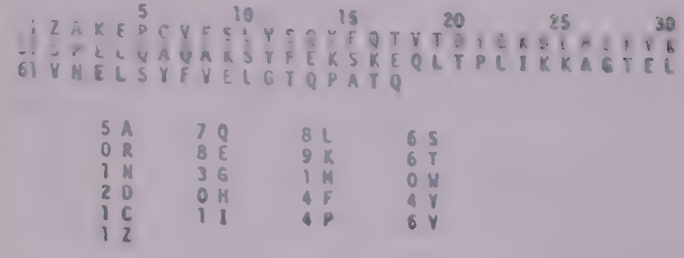

Fig. 2 — Amino acid sequence of human AII-monomer[78] The amino terminal residue is pyrrolidone carboxylic acid. The molecule is isolated as a dimer of identical chains linked by a disulphide bridge at position 6.

Apoprotein AIII[15] (*apoprotein D*[7] *or thin-line peptide*[6]) — The thin-line protein is immunologically distinct from the known proteins in the A, B, and Capoprotein families and does not react with their antibodies[15]. On the basis of amino acid composition and sedimentation equilibrium, it has a molecular weight of 19,000-20,000[15]. It contains 29 moles glucosamine per 100,000 g of protein and has all the amino acids except cysteine[7,15]. The carboxyl terminal amino acid is serine[15]. The function of the thin-line peptide has not been elucidated yet. Significantly, it is one of the constituents of the abnormal lipoprotein (LP-X) that accumulates in familial lecithin: cholesterol acyltransferase deficiency[4,84].

Apoproteins C — The apoprotein C family contains three distinct apoproteins, namely CI, CII and CIII. CI consists of a single polypeptide chain of 57 amino acid residues with a molecular weight of 6620[18,85–87]. Its carboxyl terminal amino acid is serine[85–87]. It has high lysine (16%) content as well as one residue each of methionine and tryptophan, but no cysteine, tyrosine and histidine[85–87]. The amino acid sequence for human CI has been reported (Fig. 3)[85–87].

Apoprotein CII is a single polypeptide of 95-100 amino acid residues[18]. Its molecular weight by amino acid composition has been reported to be 10,519, whereas, by sedimentation equilibrium, the value is 12,630[18]. The primary sequence has not been reported yet.

Apoprotein CIII has three polymorphic forms, CIII-0, CIII-1 and CIII-2, corresponding to a content of 0, 1 and 2 residues of sialic acid respectively in the

molecule[18,88]. The function of the carbohydrate moiety is not known. The carbohydrate is attached to threonine 74 by an O-glycosidic linkage[18,88,89]. The CIII apoproteins are composed of a single polypeptide chain of 79 amino acid residues, cysteine and isoleucine being absent[88,89]. Its primary amino acid sequence is presented in Fig. 4[88,89].

Arginine-rich apoprotein — The arginine-rich apoprotein is distinguished from all other apoproteins by its relatively high content of arginine[26,27,59]. The molecular weight of this protein is 33,000 and its amino and carboxyl terminal amino acids are lysine and alanine, respectively[36]. Though the carboxyl terminal amino acid is the same as in CIII apoproteins, they differ in amino terminal, in amino acid composition and in the carboxyl terminal amino acid sequence[36]. The arginine-rich protein exists in three polymorphic forms having the same amino acid composition and similar electrophoretic mobility, but separable by DEAE-cellulose chromatography[27].

Physiological Functions of Apoproteins

Transport of lipids — The basic function of the lipoproteins is to transport water-insoluble lipids through the plasma from sites of origin to those of storage and utilization. Lipid transport is a dynamic process: the lipoprotein particles are being constantly synthesized and catabolized, their lipid and protein components are being constantly exchanged among various lipoprotein particles, and they are interacting with the membranes and organelles of cells in the tissues[9,90]. The lipid moiety usually provides substrate to the tissues for energy requirements or can be used for the synthesis of cell membranes. The apoproteins are thought to serve primarily as determinants of the types of lipids that the particle may carry[5,6]. In addition, they may determine the receptor sites to which the particle can bind and the extent of interactions with the tissues[91]. Moreover, some of the apoproteins are known to activate or inhibit the activity of enzymes involved in the metabolism of the plasma lipids, including lecithin: cholesterol acyltransferase[4,9,92,93] and lipoprotein lipase[4,9].

Lecithin: cholesterol acyltransferase (LCAT) — Lecithin: cholesterol acyltransferase catalyzes the esterification of lipoprotein cholesterol by preferentially utilizing fatty acid from the C-2 position of lecithin[92,93]. It is secreted by liver into the plasma, where it circulates as a complex with HDL and is thought to act directly on small HDL particles[93]. The cholesterol and lecithin of HDL used for this reaction are readily replenished by equilibration of

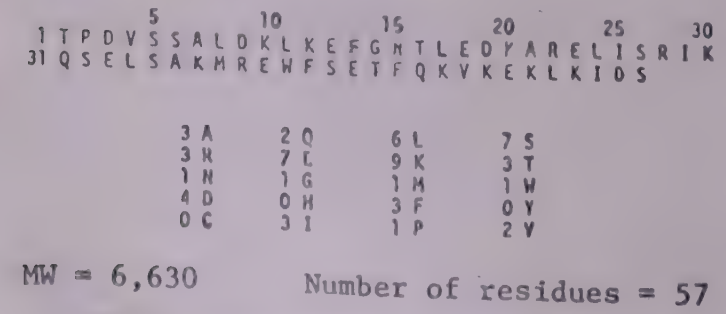

Fig. 3 — Amino acid sequence of human C-I[86]

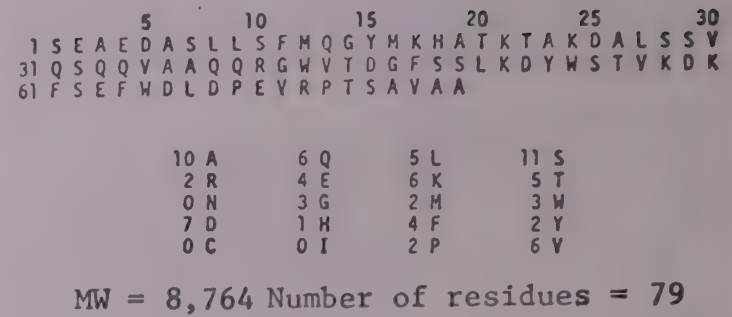

Fig. 4 — Amino acid sequence of human C-III[88][Threonine-74 is covalently bonded to one molecule each of galactosamine and galactose and 0, 1, or 2 molecules of sialic acid (designated C III-0, C III-1, or C III-2, respectively)].

these lipids among the various lipoproteins[93]. The LCAT reaction is the principal source of cholesterol esters in plasma and probably plays a significant role in cholesterol transport and homeostasis in the body[93].

LCAT activity is influenced by the acyl composition of lecithin and by many apoproteins[93]. HDL apoproteins activated a partially purified preparation of LCAT when HDL lipids were used as substrate[94]. Among the apoproteins of HDL, AI and CI enhanced LCAT activity, the extent depending on the nature of the substrate[95]. Apoprotein AI was much more effective with unsaturated than with saturated phospholipids. By contrast, CI gave similar activation with both types of phospholipids. Apoproteins AII, CII and CIII did not activate the enzyme[95]. In fact, apoproteins AII and total apo C fraction from HDL suppressed the activation caused by AI[95]. Kostner[96] has indicated that apoprotein AIII can stimulate LCAT activity, and, according to Olofsson and Gustafson[97], it may serve as a specific carrier of lysolecithin after the action of LCAT on HDL.

Lipoprotein lipase (LPL) — The lipoprotein lipase(s) hydrolyzes the plasma triacylglycerols to produce fatty acids and diacylglycerols. It is believed that during normal clearance of triacylglycerols from plasma, the enzyme is active mainly in the capillary endothelium. Very little activity is present in normal plasma, but substantial activity is found after intravenous injection of heparin[98]. The hydrolytic activity in the post-heparin plasma is derived mainly from liver and probably some from adipose tissue[99–101]. The activity released from adipose tissue is due to an enzyme different from that released from liver[100,101]. These two enzymes differ in immunological properties, inhibition by 1*M* sodium chloride or protamine sulphate and activation by specific apoproteins[102–105]. The adipose tissue enzyme is completely inhibited by protamine sulphate, sodium pyrophosphate and sodium chloride, and it is activated by HDL and apoprotein CII[102–105]. In addition to the adipose tissue and post-heparin plasma enzymes[16,102–107], CII also has been found to be a potent activator of the LPL from heart[108] and cow's milk[103,109]. The role of CII as an activator is well established, but there is no agreement on the effect of apoproteins CI and CIII on the LPL activity from different sources. Apoprotein CI was found to activate LPL from post-

heparin plasma, but not from adipose tissue or milk[102,105,109]. In contrast, Havel *et al.*[103] reported a small but measurable activation of the human and rat post-heparin plasma and rat adipose tissue enzyme at low concentrations of CI and CIII. Higher concentrations inhibited these enzymes. Bensadoun *et al.*[107] also found strong inhibition of pig adipose tissue LPL by apoproteins CI and CIII. Attempts have been made by Ostlund-Lindquist and Iverius[110] to explain these apparently conflicting findings. They have indicated that CI and CIII can inhibit or enhance the bovine milk lipase under certain conditions. At optimal concentrations of substrate and apoprotein CII for maximal activity, the CI and CIII polypeptides inhibit the hydrolase, whereas they stimulate the activity in the presence of excess substrate. Recently, Kinnunen and Ehnholm[111] have reported that human post-heparin plasma hepatic lipase is inhibited by CI, CII and CIII apoproteins.

Effect of Diseases and Diet on Apoprotein Composition of Lipoproteins

The metabolic disorders associated with plasma lipoprotein abnormalities can be divided into three main groups: (i) primary, familial or genetically determined[112–118], (ii) secondary to another disease[115–117,119], and (iii) induced by some dietary component[116]. The primary dyslipoproteinemias are hereditary and can be characterized by hyperlipoproteinemia[112] (elevation of one or more families of lipoproteins, or the presence of abnormal lipoproteins) or by hypolipoproteinemias[113] (one or more lipoprotein families are absent in the plasma or their concentrations are extremely low). The secondary hyperlipoproteinemias are usually associated with diabetes millitus[116,120], hypothyroidism[59,115,116], nephrotic syndrome[117] or obstructive liver diseases[115,117,121]. The diseases in which secondary hypolipoproteinemia results include hyperthyroidism[122,123], myocardial infarction[116,124,125] infection[116,126], anemia[127] and malabsorption of fat[128]. Some dietary factors like excess amounts of fats[116], carbohydrates[116] and orotic acid[129] also induce changes in the plasma lipoproteins and produce either hyper- or hypolipoproteinemia.

Effect of diseases — The current information about the relationship between diseases and lipoproteins is summarized in Table 5.

Effect of diet — The lipoprotein spectrum in plasma has been found to show marked changes after feeding high cholesterol diets to several species of experimental animals, namely rat[145,146], guineapigs[147,148], rabbits[59,149,150], swine[151] and monkey[152]. In most cases, the changes are characterized by the appearance of new lipoprotein species which can be distinguished from normal lipoproteins on the basis of lipid and apoprotein composition and by their density and electrophoretic mobility. Two abnormal lipoprotein species (β-VLDL and HDL_C) usually appear in the plasma of cholesterol-fed animals[152]. The β-VLDL (it has β mobility, but floats at $d < 1{\cdot}006$) fraction from these animals has many properties similar to those of β-VLDL found in Type III hyperlipoproteinemia in humans. As compared to normal VLDL, the most striking feature of β-VLDL particles from cholesterol-fed animals is the presence of large amounts of cholesterol esters and arginine-rich apoprotein[152]. The arginine-rich apoproteins present in the plasma of various animals seem to have molecular weights similar to those of their counterpart in human VLDL[152]. The intermediate density lipoproteins and LDL_2 (d,1·05-1·085, pre-β mobility, contain apoproteins AI, B and high amounts of sialic acid) fractions of cholesterol-fed monkeys also show an abnormal content of arginine-rich apoprotein[152]. The HDL_c (α_2 mobility, d 1·03–1·08, lack apoprotein B) isolated from the plasma of cholesterol-fed swine[151] and monkeys[152] also contain high proportions of arginine-rich apoprotein in addition to AI and AII. Due to the presence of large amounts of a rginine-rich apoprotein in cholesterol-rich lipoprotens, it has been suggested that this apoprotein is invilved in transport and metabolsim of cholestrol. It orobably also has some role in metabolic processes pading to hyperlipoproteinemia and atherosclerosiseassociated with the high cholesterol diets.

The effect of saturated and polyunsaturated dietary fats on the apoprotein patterns of plasma has not been reported in the literature. Diets rich in unsaturated fats lower the plasma cholesterol levels. Saturated fats have the opposite effect. Recently, Stange[153] has studied the lipid and protein composition of various lipoprotein fractions after feeding diets containing 1% cholesterol or 1% cholesterol+5% saturated fat or 1% cholesterol + 5% unsaturated fat to rabbits. They noted marked increase in the total cholesterol content of all lipoprotein fractions in the plasma of animals fed the three diets. Polyunsaturated fat did not have a lowering effect on serum cholesterol when it was fed in combination with cholesterol; rather, plasma cholesterol values were slightly higher than those in the cholesterol-fed group. The corresponding values for the cholesterol+saturated fat group were higher than for the other two groups. The changes of the VLDL and LDL fractions in the lipid composition from the three dietary groups were accompanied by changes in the polyacrylamide gel electrophoretic patterns of the apoproteins.

Feeding of orotic acid to rats produces a plasma lipoprotein pattern very similar to the one found in abetalipoproteinemia in humans, where VLDL and LDL are virtually absent in the plasma[129]. The deficiency of these lipoproteins seem to result from a blockage of apoprotein B synthesis in the liver. Small amounts of apoprotein B originating from the intestine, however, can be found in the mesenteric lymph of these animals[129]. The apoprotein patterns of the serum HDL, from orotic acid-fed rats are essentially similar to those of HDL of normal controls[154]. However, the new HDL particles (d, 1·06-1·21) secreted by the liver of orotic acid-fed rats differed from the corresponding fraction from normal rats as the ratio of arginine-rich/AI in the former group was 5 as compared to 1·6 in the normal rats[154]. These observations suggest that the HDL found in the plasma differs from the particles released from the liver and that this transformation takes place in the plasma.

TABLE 5 — LIPOPROTEIN AND APOPROTEIN PATTERNS OF PLASMA IN VARIOUS DISEASES

Disease	Changes in lipid concentration*		Lipoprotein abnormality	Apoprotein abnormality	Ref.
	Cholesterol	Triacylglycerol			
A. *Familial or essential hyperlipoproteinemia*					
Type I (deficiency in lipoprotein lipase)	↑*	↑	Chylomicrons present and markedly increased. VLDL, LDL, HDL normal or decreased.	Not documented	112
Type II	↑	↑ or normal	LDL increased, VLDL normal (Type IIA, hypercholesterolemia). LDL and VLDL increased (Type IIB, combined hyperlipoproteinemia).	Serum contains low amounts of AI with concentration of apoprotein B five times higher than normal. B has normal amino acid composition. Type IIA, LDL, has higher ratio of B/C than normal LDL_1	46, 49, 112, 130
Type III (broad β-disease)	↑	↑	Presence of β-VLDL, which floats in VLDL density but has β-mobility and abnormal lipid composition.	β-VLDL has lower C, increased B and arginine rich twice (by wt) as much as normal VLDL.	59, 61, 112
Type IV	↑ or normal	↑	Chylomicrons absent, VLDL increased, LDL normal.	VLDL contains 70-80% B, 10% A, 10-20% C. Ratio of CII/CIII decreases with increasing triacylglycerol content of VLDL.	5, 131
Type V	↑	↑	Chylomicrons present, VLDL increased.	VLDL contains 48% B, 4% A 48% C. Ratio of CII/CIII decreases with increasing triacylglycerol content of VLDL.	5, 131
B. *Familial lipoprotein deficiency*					
Abetalipoproteinemia	↓	↓	Normal chylomicrons, VLDL, LDL, absent.	Plasma: B and CIII-1 absent.	
			Presence of small amounts of abnormal lipoproteins in LDL density range.	Abnormal "LDL": contains AI, AII and C-peptides; B absent.	16, 43, 46, 113, 132-134
			"HDL" also abnormal in chemical composition and concentration.	"HDL" contains uncharacterized components in addition to apoproteins present in normal HDL. They also have lower amounts of C-III-1 and twice the amount of C I as compared to normal HDL.	
Hypobetalipoproteinemia	↓	↓ or normal	LDL decreased.	Apoprotein patterns of VLDL, LDL and HDL appear to be normal.	113, 135

(*Contd*)

TABLE 5 — LIPOPROTEIN AND APOPROTEIN PATTERNS OF PLASMA IN VARIOUS DISEASES (*Contd*)

Disease	Changes in lipid concentration*		Lipoprotein abnormality	Apoprotein abnormality	Ref.
	Cholesterol	Triacylglycerol			
Tangier disease	↓	↓ rarely normal	HDL low, presence of abnormal HDL called HDL_T	Serum: AII low and AI absent or low (only 1% of that found in normal serum). HDL_T: AI and C absent, contains mainly AII with AI/AII weight ratio of 1:12 as compared to 3:1 in normal HDL.	46, 113, 136, 137
C. *Familial lecithin: cholesterol acyltransferase deficiency*	↓ (ester form) ↑ (free form)	↑	Presence of β-VLDL, normal VLDL absent.	Plasma: A and B absent.	70 84, 93, 114, 138, 139.
			Abnormal LDL fraction contains LP (X)	LP(X): contains albumin (35%), C (65%, CI+CII+CIII). Does not react with antisera to AI and AII but gives a weak reaction with anti-α_1-lipoprotein serum, probably due to AIII.	
			Presence of abnormal HDL fraction (LP-E).	LP-E: contains apo E (probably same as arginine-rich protein).	
			Presence of abonormal "HDL" fraction with 45-60 Å size.	"HDL" contains AI, very small amounts of AII. C absent.	
D. *Secondary lipid disorders*†					
Liver diseases secondary LCAT deficiency with or without cholestasis)	↓ (ester form) ↑ (free form)		Presence of β-VLDL, normal VLDL and HDL decreased. Abnormal LDL fraction contains LP-X which does not react with antisera to LDL, has β-mobility and is a poor substrate for LCAT. It resembles LP-X described in familial LCAT deficiency and in cholesterol fed guineapigs.	β- VLDL lacks A and has lower amounts of C than normal VLDL. LP-X: contains albumin (40%), C (60%, CI+CII+CIII). AIII and AI absent.	70, 93, 115, 121, 122, 140-143.
			Presence of abnormal HDL (LP-E).	LP-E: contains apo E (probably same as arginine-rich protein).	
			Abnormal HDL present in obstructive jaundice have β-mobility.	"HDL_1": contains albumin, high proportions of "arginine-rich" and C-polypeptides particularly CIII-2, AI/AII ratio reversed as compared to normal HDL.	
Diabetes accompanied by hyperlipidemia			Similar to Type IIA or IV hyperlipoproteinemia. Triacylglycerol-enriched LDL and HDL.	Increased B in plasma and LDL fraction in Type IV+ diabetes but not in Type IIA + diabetes as compared to hyperlipidemic-non-diabetic counterparts.	120
Hypothyroidism accompanied by hypercholesterolemia	↑		Presence of β-VLDL. Elevated LDL_1 and LDL_2.	High content of arginine-rich protein in β-VLDL.	59, 115
Hyperthyroidism accompanied by hypocholesterolemia	↓		Presence of β-HDL which floats in HDL density range but has β-mobility.	"β-HDL": contains B and H_1 (probably same as arginine-rich protein).	115, 144

*The lipid concentrations higher (↑) or lower (↓) than normal values in plasma.
†Plasma lipid concentrations depend on the type of dyslipoproteinemia accompanying the primary disease.

The feeding of high carbohydrate diets to humans produces changes in the concentration and composition of the plasma VLDL, LDL and HDL fractions[155]. All three fractions become enriched with triacylglycerol relative to cholesterol. In these subjects, the apoprotein AI content of the plasma is decreased, whereas total apoprotein B levels are normal. However, the density distribution of apoprotein B changed. The ratio of apoproteins CII/CIII in VLDL was about 1·5 times higher in carbohydrate-fed subjects than in the controls. Similar changes in the relative proportion of apoprotein C have also been observed in the VLDL of rats fed high carbohydrate diets[156], Zucker fatty rats[157] or in the rats made diabetic[158].

The various conditions described above for diseases and diet show a variety of lipoprotein patterns in plasma. In some of these conditions, there is a change in the concentrations of normal lipoproteins, whereas in others there is an alteration in the lipid and apoprotein compositions as well as in concentration of a lipoprotein class. Based on our present knowledge, it cannot be determined how changes in apoprotein patterns are related to the various types of dyslipoproteinemia. The changed apoprotein patterns could cause dyslipoproteinemias by influencing the catabolism of the lipoproteins. Alternatively, the changes may be a reflection of other metabolic abnormalities in the body which result in synthesis and secretion of altered lipoprotein particles.

Current Trends in Lipoprotein Research

Lipoprotein research is in a phase of rapid growth. Most of the efforts are being directed to understand the basic biochemical mechanisms by which abnormalities in lipoproteins cause atherosclerosis. The major approaches adopted to achieve this goal are outlined below: (i) Use of physical and chemical techniques to study protein-lipid interactions and to gain information on the organization of lipids and proteins in the lipoprotein molecule[9–12]. The rapid advances in the study of lipid-protein interactions in the field of membrane biology has provided the needed impetus for such studies with lipoproteins. (ii) Studies on the synthesis, secretion and catabolism of lipoproteins under normal and various pathological and physiological conditions in humans and many experimental animals are being undertaken[9,10]. Sophisticated equipment and techniques for the analysis of protein and lipid moieties are being employed. Moreover, information on the distribution and composition of lipoproteins in the plasma of various animals may provide insights into the structure and function relationship of these macromolecules. For example, the human LDL fraction is almost entirely made of apoprotein B, whereas the corresponding fraction from rats[154] and mice[159] contains appreciable amounts of other apoproteins besides B. Apoprotein AII exists solely in monomeric form in the plasma of rhesus monkey[80], gibbons[10], baboons[80], rat[160,161], rabbit[80], cow[80] and dog[80,162]. In man and chimpanzee[163], it is present as a dimer. The relationship of these species differences to the lipid carrying capacity of the lipoproteins is yet to be elucidated. (iii) Tissue culture systems hold great potential for enhancing our understanding of how the alterations in lipoprotein composition affect lipid uptake and regulate metabolic activity of cells[164–169]. Recently, Brown and Goldstein[91] have demonstrated potential usefulness of this approach in explaining the biochemical defects in familial hypercholesterolemia. Using human fibroblast cultures, they showed that cells from subjects with this disease have fewer LDL receptors than normal. This defect leads to increased cholesterol synthesis because of the cells becoming less sensitive to feedback regulation by serum cholesterol levels.

Summary

The protein part of the plasma lipoproteins contains several distinct apoproteins. So far, four major apoprotein families designated as apo A (contains AI, AII), apo B, apo C (contains CI, CII, CIII-0, CIII-1, CIII-2) and apo D have been isolated and characterized. Many of these proteins are common to the chylomicron, very low-, low –, and high density lipoprotein fractions. The primary amino acid sequence of apoproteins AI, AII, CI and CIII from human plasma has been determined. Their secondary and tertiary structures and the role of lipids in their conformation and stability remain to be elucidated. Very little information is available on the synthesis, secretion and metabolic fate of the protein moiety of the lipoproteins. The specific functions of the individual apoprotein components in the structure and metabolism of lipoproteins are largely unknown, though some general biochemical functions have been ascribed to some of the apoproteins. For example, apoprotein B, a major component of VLDL and LDL, appears to be essential in the secretion of these lipoproteins and in their ability to carry triacylglycerols. Apoproteins AI and CI enhance and apoprotein AII may inhibit the activity of lecithin-cholesterol acyltransferase, thereby regulating the formation of cholesterol esters and the catabolsim of lipoproteins in the plasma. Similarly, apoprotein CII activates and CIII-1 and CIII-2 inhibit lipoprotein lipase, the enzyme which plays an important role in clearing chylomicrons and VLDL from the plasma. Changes in the plasma apoprotein pattern in certain diseases and physiological states are associated with the presence of altered lipoproteins in the plasma. These have an abnormal lipid composition and metabolic fate.

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Vitamin Deficiency in Man — Recent Studies on Fat-Soluble Vitamins, Thiamin, Riboflavin, Pyridoxine & Vitamin C

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SINCE the discovery of vitamins towards the beginning of this century, a great deal has been learnt regarding the clinical pathology of vitamin deficiencies and the biochemical functions of B-complex vitamins. Vast gaps, however, exist in our knowledge of the biochemical functions of fat-soluble vitamins and vitamin C. With the exception of a few clinical symptoms, such as night blindness due to vitamin A deficiency, a correlation between clinical features and biochemical functions cannot be established. For instance, it is rather puzzling that the deficiencies of vitamins, such as thiamin, riboflavin and niacin (all concerned with carbohydrate metabolism and energy production), should lead to such diverse clinical signs.

Vitamin deficiency in man can arise through the following reasons: (a) inadequate intake due to poverty, ignorance, lack of incentive, anorexia, dental problems, apathy, and chronic diseases; (b) poor digestion and absorption due to absorptive disorders and parasitic infections; and (c) increased requirement associated with certain physiological states like rapid growth, pregnancy (foetal requirement as well as hormonal effects), drug and hormone therapy. Recently, certain vitamin-responsive inborn errors requiring pharmacological doses of vitamins for their treatment have been discribed[1].

Due to the enormous scope of the present subject as well as the recent reviews on pellagra[2], folic acid[3] and cobalaminals[4], this review has been confined to only fat soluble vitamins, thiamin, riboflavin, pyridoxine and vitamin C.

VITAMIN A

Pathogenesis & prevalence of vitamin A deficiency — In man, the clinical manifestations of vitamin A deficiency are predominantly ocular. The term 'xerophthalmia' is used to indicate all eye lesions of vitamin A deficiency, such as night blindness (rod dysfunction due to impaired synthesis of rhodopsin), conjunctival and corneal changes. In children, Bitot spot (silvery grey foamy plaque on the conjunctiva) is invariably due to vitamin A deficiency, though in adults its etiology is uncertain. Night blindness and conjunctival xerosis can be reversed with vitamin A treatment. Corneal lesions are more serious and can be reversed only in early stages. Once colliquative necrosis and keratomalacia set in, the changes are irreversible resulting in loss of vision. In a recent experimental study in human volunteers it was shown that anaemia can occur through lack of vitamin A in man[5].

The commonest cause of vitamin A deficiency is dietary inadequacy, particularly in populations depending on carotenoid precursors rather than preformed vitamin A as the dietary source of the vitamin. Diarrhoea, infections and worm infestations may aggravate the condition. The absorption of [11, 12—$^3H_2$] retinyl acetate has been reported to be impaired in children suffering from acute infections[6]. Vitamin A deficiency is often associated with protein calorie malnutrition (PCM) in children. Several studies on animals have suggested that protein deficiency affects utilization of vitamin A and β-carotene. However, the data from studies on man have given conflicting reports. In children suffering from PCM, improvement in plasma vitamin A has been observed with protein supplements alone[7]. Protein deficiency may impair the synthesis of retinol binding protein and thus affect the mobilisation of the vitamin from the liver. The increase in serum vitamin A following oral administration of vitamin A to children suffering from PCM has been found to be poor by some workers, but not by others. The presence or absence of fat has been reported to make a lot of difference to the absorption of vitamin A and may explain some of the conflicting reports[8]. Several workers have shown that, in children, PCM does not affect the utilization of β-carotene. This is supported by the observation that in African children habituated to red palm oil, which is rich in β-carotene, vitamin A deficiency is not seen despite severe PCM[9].

Severest forms of deficiency are seen in children in the age group 1-5 years. During the first six months, vitamin A needs are met exclusively from breast milk. The concentration of vitamin A in the breast milk of well-nourished mothers is reported to be 50-60 μg/100 ml, whereas that of undernourished mothers is 20-25 μg/100 ml. While some workers have found an increase in breast milk vitamin A concentration following supplementation of mothers, others could not observe a similar beneficial effect[8]. Malnourished mothers have very low liver reserves and it is possible that a good part of the supplement goes to build the maternal tissue reserves.

Prevention and treatment of vitamin A deficiency — Foods rich in vitamin A are costly, but green leafy vegetables are a cheap and good source of β-carotene. Due to prejudices, this rich source is eliminated from the diets of pre-school children. Until such time that the attitudes change, immediate and rapid measures have to be taken. Two approaches, viz. fortification of common food items with vitamin A and administration of massive doses of the vitamin at suitable intervals, have currently been tried for the prevention and treatment of vitamin A deficiency. Attempts to fortify salt have proved unsatisfactory. In Guatemala, fortification of sugar appears to be promising[8]. Since vitamin A is unique in its capacity to get stored in the liver, massive dose aproach seems logical. After numerous trials, currently children in several States of India and in other countries like Indonesia and Bangladesh receive 200,000 I.U. of vitamin A twice a year. Results hitherto obtained seem encouraging, though some doubts have also been expressed.

To effect rapid increase in blood levels of vitamin A in patients with ocular lesions, parenteral administration of water-soluble vitamin A is recommended. Solutions in oil have been found to be effective orally[8].

Transport, metabolism and biochemical functions of vitamin A — Though research on the transport, metabolism and biochemical functions of vitamin A has been rather slow, important leads have been obtained in the last decade, particularly on transport of retinol. The subject has been extensively discussed[11,12].

Transport — Vitamin A is derived as preformed vitamin or as carotenoid precursors, such as β-carotene. In either case, retinyl esters are formed in the intestine and are absorbed into the body via the lymphatic pathway in association with lymph chylomicrons. The chylomicron vitamin A complex is removed from the circulation by the liver. Goodman and associates have shown that the vitamin is mobilized from the liver as free alcohol, retinol, bound to a specific transport protein, retinol-binding protein (RBP), and carried to different tissues, to meet their metabolic needs[10]. RBP contains a single polypeptide chain with a molecular weight of 21,000 and forms 1:1 molar complex with retinol. Under physiological conditions, more than 90% of the plasma RBP is complexed to thyroxine binding prealbumin[11].

Plasma vitamin A, RBP and prealbumin show a significant fall in their contents in liver diseases, hyperthyroidism, cystic fibrosis and in protein calorie malnutrition[10,12,13]. In chronic liver disease, the serum levels of RBP and vitamin A are elevated, but prealbumin levels remain normal. Since RBP is a protein of small size, it is possible that free RBP is filtered rapidly through the glomerulus. Though very small portion of total RBP circulates in free form, it is apparently enough to permit a significant amount of RBP to be filtered by the glomeruli and get metabolized by the kidneys each day. Patients with impared tubular function and tubular proteinuria, as well as chronic cadmium poisoning excrete large amounts of RBP in the urine[11].

Apart from solublizing retinol, RBP may also serve to prevent retinol from exerting its surface active properties in the body in a generalized way, and regulate the delivery of adequate amounts of retinol to specific sites. In hypervitaminosis A, the concentrations of plasma RBP and prealbumin are not elevated though retinol levels are[15]. It is possible that vitamin A toxicity occurs when excessive amounts of vitamin A are presented to the cell membranes in association with lipoproteins rather than specifically bound to RBP.

To elucidate the mechanism of uptake of retinol bound to RBP by the tissues, Peterson and associates have used *in vitro* system of dispersed monkey small intestinal cells[11]. When RBP bound to retinol was covalently linked to Sepharose and incubated with the cells of small intestine, there was significant uptake of retinol but not of RBP. When ^{125}I labelled RBP was used as the donor of retinol, no evidence of physical binding of RBP to the cells could be obtained. These experiments suggest that cells extract retinol from RBP without taking up the protein moiety. A rapid transitory binding of the protein to the cell surface may, however, occur, since the cell surface seems to recognize preferentially the protein-ligand complex.

Proteins capable of binding retinol *in vitro* were identified by Chytill and colleagues in the high speed supernatant fraction of homogenates of several tissues[10]. The presence of a specific retinoic acid binding protein has been reported in malignant human tumours of breast and lung, but not in normal tissues from the same organ of the same patient. This protein can bind several analogues of retinoic acid as well[15]. In view of the antitumour effects of retinol and its analogues in several species of experimental animals, it has been suggested that the control of growth and diffrentiation of cells may involve specific binding proteins for retinol and retinoic acid[15]. In a five year follow-up mail survey, the incidence of pulmonary carcinoma was found to be negatively correlated with vitamin A intake[16].

Mode of action — Except its role in night vision, the mode of action of retinol at the cellular level continues to be an enigma. Recent work of Peterson and associates has shown that retinol phosphate may have a direct role in glycosylation reactions of glycoprotein synthesis[11]. The role of glycolipids containing polyprenol phosphate sugars had earlier been demonstrated in bacterial cell wall. One of the lipid moieties in such compounds was identified as dolichol. The formation of retinyl phosphate mannose and retinyl phosphate galactose has been demonstrated in the membrane fractions of the liver and mastocytoma[11]. Urinary excretion of sulphated mucopolysaccharides has been found to diminish in vitamin A-deficient children[17].

Retinol seems to be essential for maintaining the integrity of membranes. Both hypo and hypervitaminosis A lead to lysosomal damage. The urinary excretion of two lysosomal enzymes, arylsulphatase and acid phosphatase, was found to diminish following the administration of vitamin A to children having ocular lesions of vitamin A deficiency[18]. Children with kwashiorkor also excrete higher than normal activity of lysosomal enzyme arylsulphatase[19]. Since vitamin A deficiency and PCM are invariably associated in Indian children, it is difficult to dissociate their effects.

Recently, vitamin A deficiency in rats was reported to retard hepatic cell regeneration[20] and delay the cell cycle of jejunal crypt cells[21]. Whether the observed effects are typical of vitamin A deficiency or would have been observed in other deficiencies as well needs to be checked. Hypervitaminosis A during organogenesis has been shown to be teratogenic in anmials. In a case study, excessive intake of vitamin A during pregnancy was reported to result in the birth of a child with congenital anomalies[22].

Metabolism — Several metabolites of retinol and retinoic acid have been detected in urine[24,25]. The structures of these metabolites in rat and man tend to be similar. A protein bound retinol or a metabolite of retinol (distinct from retinol bound to RBP) has been found in the urine of rat and man. Its excretion was found to diminish in vitamin A-deficient rat[25].

TABLE 1 — DAILY ALLOWANCES OF NUTRIENTS FOR INDIANS
(Recommended by the Nutrition Expert Group of the ICMR in 1968)

Group	Particulars	Vitamin A		Thiamin mg	Riboflavin mg	Nicotinic acid, mg	Ascorbic acid, mg	Folic acid, μg	Vitamin B_{12}, μg	Vitamin D I.U.
		Retinol μg	β- carotene μg							
Man	Sedentary work	750	3000	1·3	1·3	16	50	100	1	
	Moderate work			1·4	1·5	19				
	Heavy work			2·0	2·2	26				
Woman	Sedentary work	750	3000	1·0	1·0	13	50	100	1	
	Moderate work			1·1	1·2	15				
	Heavy work			1·5	1·7	20				
	Pregnancy (Second half of pregnancy)	750	3000	+0·2	+0·2	+2	80	150-300	1·5	
	Lactation (upto one year)	1150	4600	+0·4	+0·4	+5	80	150		200
Infants	0-6 months	400	—				30	25	0·2	
	7-12 „	300	1200							
Children	1–3 years	250	1000	0·6	0·7	8				
	4–6 years	300	1200	0·8	0·8	10				
	7–9 years	400	1600	0·9	1·0	12				
	10–12 years	600	2400	1·0	1·2	14	30–50	50–100	0·5–1·0	
Adolescents	13-15 yr Boys	750	3000	1·3	1·4	17				
	Girls			1·1	1·2	14				
	16–18 yrs Boys	750	3000	1·5	1·7	21				
	Girls			1·1	1·2	14				

Biochemical assessment of vitamin A status — Vitamin A deficiency in man leads to rapid depletion of liver reserves and fall in plasma vitamin A concentration. Serum vitamin A levels below 10 μg/100 ml are strongly suggestive of deficiency in all age groups. Plasma vitamin A above 20μg and carotene above 40 μg are accepted as low risk values[26]. For pregnant women, values above 40 μg in the second trimester and values above 80 μg in the third trimester are accepted as low risk values[26]. Serum level less than 20 μg is believed to indicate little or no liver reserves.

Despite many drawbacks, serum vitamin A is the only method available for assessing vitamin A status in man. Several diseases and drugs affect serum vitamin A levels[26]. The levels tend to be depressed in subjects with febrile condition, chronic infection, liver diseases and sprue. Children with cystic fibrosis have low levels of serum vitamin A due to a defect in the mobilization and transport of retinol from the liver rather than due to depleted tissue levels. Women ingesting oral contraceptives tend to have higher serum levels[26].

Vitamin A requirement in man — According to a study conducted on human volunteers by the staff of the University of Iowa, USA and U.S. Army Medical Research and Nutrition Laboratory, Denver[27], an adult man appears to require at least 600 μg retinol per day to prevent or cure the eye changes and perhaps more to reverse the cutaneous lesions. The requirement of β-carotene is approximately 1200 μg/day. These levels, however, would not necessarily support optimal levels of plasma vitamin A. Intakes of 1200 μg/day of retinol or 2400 μg/day of β-carotene seem to ensure plasma vitamin A levels above 30 μg/100 ml, which are judged desirable. The recommendations made by the Indian Council of Medical Research are given in Table 1[28].

VITAMIN D

Vitamin D was identified as the antirachitic factor in 1922. Its role in calcium transport from the intestine and mobilisation from the bones has been known since a long time. Natural vitamin D_3 (calciferol) is derived from animal products and from the action of ultraviolet light on the skin. It is the predominant form of the vitamin in circulation, even in people who receive foods fortified with ergocalciferol. Skin is the most important source of the vitamin for man[29].

The daily requirement of vitamin D is not more than 400 IU and it may be as low as 70 IU[30]. It should be possible to obtain this amount through exposure to sunlight. Yet, despite the abundance of sunshine in tropical counries, rickets is seen in children whose dietary in take of vitamin D is low. Vitamin D deficiency is seen in premature infants, in elderly women (where it is related to the estrogen level), in immigrants to UK, in strict vegetarians and in those whose intake of animal fat is low[31]. Other factors etiological in the development of vitamin D deficiency are defective intestinal absorption, liver derangement[9] renal abnormalities and abnormal parathyroid activity. High incidence of rickets among Asian immigrants to UK is believed to be due to high dietary phytate and vitamin D deficiency[32].

The pathology of vitamin D deficiency in man is confined to the skeleton. In infants and children up to the age of fusion of the epiphysis, the deficiency leads to rickets, while in the adults osteomalacia occurs.

Many disorders resembling rickets but which respond to very high doses of the vitamin or not at all (referred to as vitamin D resistant rickets) have been observed[1,3]. Recently, two cases of magnesium-dependent, vitamin D-resistant rickets were reported from Hyderabad[33]. Though florid rickets is rare in PCM, particularly in kwashiorkor, biochemical and radiological rickets in all forms of PCM was recently reported[34].

In many temperate countries with poor sunshine, food items such as milk are fortified with irradiated ergosterol. Generally ergosterol is used in the treatment of rickets and osteomalacia. In view of the recent knowledge regarding the metabolism of vitamin D to functionally active hydroxylated metabolites, the usefulness of such metabolites in the treatment of rickets, particularly the resistant types, is currently being investigated. 25-Hydroxycholecalciferol (25-HCC) has been found effective in anticonvulsant hypocalcemia[35]. 1,25-Dihydroxycholecalciferol (1,25-DHCC), the biologically active metabolite of the vitamin, has been found to be effective in renal rickets[36]. Since 1,25-DHCC is very expensive to synthesize, Schnoes *et al.*[37] have looked for suitable synthetic analogues and found that 1-α-hydroxyvitamin D_3 is a safe and effective substitute for the kidney hormone 1,25-DHCC. Clinical efficiency of 1-α-hydroxyvitamin D_3 in osteomalacia has been reported[38].

Metabolism and biochemical functions of vitamin D — The present era of vitamin D research which has led to the discovery of the kidney hormone 1,25-DHCC began in 1964, when Fraser and Kodicek discovered a polar metabolite of vitamin D with some biological activity. Research carried out in the laboratories of DeLuca and associates in Wisconsin, USA, Kodicek and associates in Cambridge, UK, and Norman and associates in Riverside, California, USA, in the last decade, has shown that the biological activity of vitamin D depends on its conversion to the kidney hormone 1,25-DHCC. The numerous reports on this subject have been reviewed[39-41]. Cholecalciferol is converted to 25-HCC, which is the predominant circulating form of the vitamin, attached to a specific globulin in the plasma. It was thought that 25-HCC can be formed only in the liver, but it has now been shown that this metabolite can be formed in the kidney and the intestinal cells as well[42]. 25-HCC is further metabolised to 1,25-DHCC in the kidney. 1,25-DHCC acts as a typical steroid hormone regulating DNA transcription in the intestinal cells, including the synthesis of a specific messenger RNA, which, in turn, is responsible for the synthesis of a calcium binding protein first described by Wasserman and collegues[43]. Since the inhibitors of protein synthesis inhibit the action of 1,25-DHCC in the bone as well as the intestine, it is possible that a similar carrier protein is induced in the bone also[44].

The production of 1,25-DHCC is regulated by feedback control either directly or indirectly by serum calcium and serum phosphorus concentrations. The hypocalcemic regulation is mediated by the parathyroid glands. The hypophosphatemic stimulus is independent of the thyroid or the parathyroid glands[39]. When the calcium and phosphorus levels are adequate, the formation of the 1,25-DHCC form ceases and 24, 25-DHCC is produced[39]. The metabolite is further converted to 1,24, 25-trihydroxycholecalciferol, which stimulates intestinal calcium transport at a slow rate but has no effect on bone calcium mobilization, or phosphate transport reactions. A number of vitamin D-resistant bone diseases may be related to defective vitamin D metabolism[39,40]. Recently, it has been shown that premature infants have a decreased rate of 25-hydroxylation of vitamin D_3[45]. This may be etiological in the development of vitamin D deficiency in premature infants.

According to a recent study in rats, the potency of vitamin D declines in the absence of adequate zinc in the diet[46]. Prednisolone has been reported to interfere with vitamin D action on the intestinal transport of calcium and phosphorus[47].

Biochemical assessment of vitamin D status — A suitable method for biochemical evaluation of vitamin D deficiency is hitherhto not available. Serum alkaline phosphatase is raised in rickets[26]. But this test is not specific, since alkaline phosphatase is elevated in other diseases and depressed in PCM. Rickets is also associated with low serum phosphorus levels and sometimes low serum calcium levels[26]. In recent years, plasma concentration of 25-HCC is being used as an estimate of vitamin D status in man.

Vitamin D requirement for man — Vitamin D requirement for man is not established. The Indian Council of Medical Research has recommended 200 IU for all age groups (Table 1). Pregnancy does not modify 25-HCC levels in plasma and no additional supplements may be required in pregnancy[48].

VITAMIN E

Pathogenesis, prevalence and treatment of vitamin E deficiency — Vitamin E deficiency in animals leads to a variety of disorders which include reproductive failure, disorders of liver, blood, brain and cardiovascular system and myopathies[49]. The symptoms vary with the species of the animal. Experimental tocopherol deficiency in humans was found to produce very little pathological disturbance. There was diminished red cell survival, but no anaemia[50].

Vitamin E deficiency in older children and adults is rare. Placental transfer of vitamin E is very poor and hence new-born infants tend to be susceptible to vitamin E deficiency. Human breast milk, however, is a good source of the vitamin, but cow's milk is not. Several reports on hemolytic anaemia in premature infants due to vitamin E deficiency have appeared[51].

The vitamin E requirement of breast fed babies is less than that of bottle fed babies, because many proprietary milk formulae have considerably higher amounts of unsaturated fatty acids than breast milk, and vitamin E requirement is related to the unsaturated fat in the diet[52]. According to one report, iron supplements aggravate hemolytic anaemia due to vitamin E-deficiency in infants, since iron catalyzes

the oxidative breakdown of red cell lipids[52]. This, however, needs confirmation.

Some of the aspects of vitamin E nutrition in man were reviewed recently[31]. Almost 72% of the oral dose is generally absorbed from the gastrointestinal track. The absorption is not affected by the level of body stores, nor the extent of fat intake, but depends on the presence of bile in the duodenum and is influenced by the properties of medium and long chain triglycerides. Biochemical evidence of low tocopherol levels has been observed in sprue, celiac disease, in fibrocytic disease of pancreas, and in post-gastrectomy patients. Very low levels are also seen in α,β-lipoproteinaemia, perhaps due to transport failure[31,52].

Pathological changes, such as ceroid deposits, creatinuria, necrosis of striated muscle, anaemia and lipofucin in myocardiac muscle have been reported after prolonged vitamin E deficiency, but a casual relationship between these changes and vitamin E deficiency is not established[31]. In children with defective absorption, a dose of 100 mg per day of the vitamin is given to correct the biochemical abnormalities and restore the plasma levels.

Vitamin E therapy has been tried in the treatment of intermittent caludication, stasis, ulcers, ischemic heart disease and in hemolytic anaemia in premature infants, with some degree of success. It is now recommended that 10 mg of vitamin E should be given to premature infants on artificial feeds from the tenth postnatal day onwards. Vitamin E supplements have been found to have no effect on the physical performance of swimmers.

Vitamin E is commonly administered as α-tocopherol acetate (TA) which is fat soluble. In a recent study[53], the therapeutic effect of a new water-soluble preparation α-tocopherol polyethylene glycol 1000 succinate (TPGS) was compared with that of TA in premature infants. The water soluble form was better absorbed and showed a better response.

Biochemical role — A unified theory regarding the mode of action of vitamin E is hitherto not available. Opinion is divided between those who feel that both vitamin E and tocopherol act as antioxidants[54] and others who maintain that vitamin E has a definite biochemical function[55]. Vitamin E deficiency has been reported to diminish the activities of the enzymes involved in heme synthesis[56]. While this claim from a single laboratory has not been confirmed, it does provide a biochemical explanation for the vitamin E deficiency anaemia seen in some species.

The recent discovery of Rotruck *et al.*[57] that selenium is a constituent of the enzyme glutathione peroxidase has helped to realize that selenium and vitamin E may exert their antioxidant effects through independent mechanisms. According to Diplock[58], vitamin E stabilizes cellular and subcellular membranes by complexing with membrane phospholipids, and protects the oxidant-sensitive regions of membrane-associated proteins that contain selenide. In vitamin E deficiency, there is functional disturbance of membrane associated enzymes[58].

Biochemical assessment of vitamin E status — Vitamin E status in man is assessed by measuring plasma tocopherol concentration or by measuring the susceptibility of erythrocytes to *in vitro* hemolysis by an oxidant[26]. Various erythrocyte hemolysis tests have been developed using hydrogen peroxide, dialuric acid or isotonic saline phosphate buffer as the hemolyzing agent. Hydrogen peroxide erythrocyte hemolysis test has been used most commonly. The measurement of blood-cell fragility with a complicated instrument called a Fragiligraph for evaluating vitamin E nutrition status has also been proposed[26].

Bieri and Poukka reported the red cell and plasma α-tocopherol concentrations of normal subjects to be 230 ± 13 μg/100 ml and 984 ± 91 μg/100 ml respectively[59]. The average serum tocopherol levels of adult males and females in Bangladesh tended to be lower, being 760 and 730 μg/100 ml respectively[60]. It has been suggested that vitamin E deficiency may be associated with PCM. Red cell hemolysis does not occur till plasma tocopherol levels fall below 500 μg/100 ml[26]. Hemolysis test is simpler than the measurement of plasma tocopherol.

Tocopherol requirement — Vitamin E requirement in man is not established. It is related to the unsaturated fat content in the diet. The present day trend is towards consumption of more unsaturated fat[61]. It has been recently suggested that dietary allowance of vitamin E should be phrased as the quantity of vitamin E activity to be measured per g linoleate in 100 g adipose tissue fatty acids. A recommendation of 0·16 IU vitamin E/g linoleate in 100 g adipose tissue fatty acids has been made tentatively[61].

VITAMIN K

Vitamin K is required for the synthesis of blood clotting factors, II (prothrombin), VII, IX and X. Its deficiency in man and animals leads to disorders of the blood clotting mechanism. In mild deficiency, there is delayed clotting. Severe deficiency manifests itself as haemorrhagic diathesis.

Some of the clinical and nutritional aspects of vitamin K deficiency in man were reviewed recently[31]. Dietary deficiency leading to clinical symptoms is very uncommon in adults, but it can arise in malabsorption diseases and ulcerative colitis. Deficiency symptoms may also arise in severe liver disease and following the use of antibacterial agents which reduce intestinal bacterial synthesis of the vitamin, or following the use of anticoagulants like coumarin and indanedione which are competitive inhibitors of vitamin K.

Hypovitaminosis K is often seen in new-born infants. Poor placental transfer of the vitamin, low levels of phylloquinone in milk and negligible intestinal biosynthesis may be causative factors. The order of disappearance of blood clotting factors in vitamin K deficiency depends on the half life of each[31].

The daily requirement of vitamin K is not established. The requirement is considerably less than 1 mg per day and according to the present evidence it may be 20-100 μg per day in adults and less than 10 μg in children.

Measurements of prothrombin and clotting times are employed for detecting bleeding disorders. If vitamin K deficiency exists, administration of the vitamin should correct the abnormal prothrombin or clotting time.

Biochemical mode of action — Administration of vitamin K antagonists like coumarin to humans and cattle leads to the appearance in the plasma of abnormal prothrombin (a precursor), which is antigenically active but biologically inactive in prothrombin bioassay systems. Unlike the normal prothrombin, the abnormal prothrombin does not bind Ca^{2+} (ref.62). A low molecular weight Ca^{2+} binding peptide containing γ-carboxyglutamic acid residues has been isolated from the normal but not the abnormal prothrombin[62]. In the prothrombin molecule, ten glutamic acid residues in the first 42 amino terminal residues are modified in this manner, and it is believed that vitamin K is involved in the γ-carboxylation of specific glutamic acid residues of the prothrombin precursor produced in the liver[62–64]. While the exact mechanism of action of Ca^{2+} in blood clotting is not known, it is believed to be essential for binding of vitamin K-dependent proteins to phospholipid surfaces. The phospholipid binding sites are present on the NH_2-terminal fragment of prothrombin (fragment I) which also contain the Ca^{2+} binding sites and the peptide bearing carboxyglutamic acid residues. Esmon *et al.*[65] provided evidence to show that the Ca^{2+}-dependent binding of prothrombin to phospholipids occurs only in the presence of γ-carboxyglutamic acid[65]. Very recently, Stenflo[66] identified and purified a new glycoprotein containing γ-carboxyglutamic acid residues from bovine plasma. Its physiological function is not known.

The potential role of vitamin K in bone development is suggested by the recent observation that γ-carboxyglutamate may be present in bone matrix[67].

VITAMIN C

Severe vitamin C deficiency in man leads to scurvy. Since ascorbic acid is very sensitive to cooking and processing, it is almost absent from tinned fruits and dried vegetables. Scurvy is seen in all age groups,but is very rare in new-born infants and more common in infants during the second year, when growth is rapid. It is a disease of artificially fed infants[68]. Scurvy is occasionally seen in adults when the diet is severely restricted due to disease, food fads or other reasons. The presenting symptoms are irritability, tenderness of the legs and pseudoparalysis involving the lower extremities. Haemorrhagic manifestations may occur in the gums, skin, mucous membranes and soft tissues. Anaemia is very frequent in scorbutic infants, though specific haematological response to ascorbic acid alone is not seen. Costochondirial beading is always seen. In adults, scurvy manifests as weakness, fatigue, listlessness, loss of appetite and haemorrhages deep in the muscle, in joints and gums. Wound healing is delayed. Repeated infections are common[68]. Large segments of the population, including smokers and aged men and women on oral contraceptives have been shown to have low levels of ascorbic acid[69]. Ascorbic acid concentration of leucocytes of many apparently normal subjects in Hyderabad has been found to be only 50-60% of the fully saturated value[70]. However, scurvy is not a major nutritional problem in India. Milder forms of vitamin C deficiency contributing to repeated infections, etc. may exist. Petechial haemorrhages are often seen in milder grades of vitamin C deficiency.

Metabolism — Newer developments in vitamin C metabolism have been reviewed recently[71,72].

Most animal species can synthesize vitamin C. The exceptions are the primates, including man, guineapigs, fruit eating bats, some fish and supposedly red-vented bulbul in India. Recently, some insects were also shown to lack ascorbic acid synthesizing capacity[73]. The biosynthetic pathway is: D-glucose→ D-gluconolactone → L-glucono-δ-lactone → L-ascorbic acid. In the species requiring ascorbic acid, the missing step is the final step, since they lack the enzyme L-gluconolactone oxidase.

In man, absorption of ascorbic acid occurs mainly from the proximal part of the small intestine by a non-passive saturable process[71]. Dehydroascorbic acid (DHA) being more lipid soluble is the preferred form of transport across the cell membrane. According to Sorell *et al.*[74] the penetration of ascorbic acid into red cells is better *in vitro* than *in vivo*. This may be due to avid uptake of ascorbic acid by other tissues *in vivo*. The *in vitro* uptake was similar, when the erythrocytes were suspended in saline or plasma[74]. However, Mohanram and Srikantia[75] have reported that subjects differ widely in their ability to take up ascorbic acid *in vitro*. They proposed the presence of factors both in the intestine and the leucocytes which would influence the ultimate concentration of ascorbic acid in leucocytes. In the blood, ascorbic acid is almost exclusively present in its reduced form. The uptake of ascorbic acid by tissues is an energy dependent Na^+ sensitive process, whereas the uptake of DHA follows the principle of passive diffusion[71].

The highest concentration of ascorbic acid is found in glandular tissues, such as pituitary gland, adrenals, corpus luteum and salivary glands. The concentrations in the muscle is very low.

The metabolism of ascorbic acid shows species difference. Thus, while carbon dioxide is an end product of ascorbic acid metabolism in the guineapig, it is not formed in man[71,72]. Ingested ascorbic acid enters the body pool and is excreted as urinary ascorbic acid, DHA, oxalate and a number of other metabolites of which only ascorbate-2-sulphate (AS) has been identified. 2,3-Diketogulonic acid is always present in urine, but it is not established if it is a natural metabolite or an artefact.

The sulphation of ascorbic acid by an ascorbic acid sulphotransferase was reported recently in rat liver and colon hemogenates[76]. Vitamin A deficiency had no effect on ascorbic acid sulphation *in vitro*.

The turnover half-time of ascorbic acid in healthy male volunteers was around 20 days, when the intake of ascorbic acid was about 100 mg/day[72]. After administration of labelled ascorbic acid, about 44% appeared as oxalic acid, 20% as unchanged ascorbic acid and 2% as DHA. The metabolic conversion of ascorbic acid to oxalate does not contribute to the increased urinary excretion of oxalate in primary oxaluria, though very high doses of ascorbic acid may raise the urinary oxalic acid excretion.

Functional role — The biochemical role of ascrobic acid is still a mystery, though several functional defects have been reported in scorbutic animals and man. The vitamin is believed to be intimately connected with phenylalanine and tyrosine metabolism. In a study designed to understand the functional significance of low levels of leucocyte ascrobic acid seen in many Indians, it was observed that at concentrations below 6 $\mu g/10^8$ cells in children and 4 $\mu g/10^8$ cells in adults, there is increased urinary excretion of tyrosyl derivatives, *p*-hydroxyphenyl pyruvic acid and *p*-hydroxyphenyl lactic acid, though clinical scurvy is not evident[77].

Ascorbic acid is believed to facilitate iron absorption and utilization. In human volunteers, ascorbic acid administered orally or parenterally was found to raise serum iron and increase iron turnover[78]. Anaemia is an associated feature of scurvy. Very often the picture is megaloblastic. In a recent case study of a patient with scurvy, urinary folate was mainly 10-formyl tetrahydrofolic acid. After treatment with vitamin C, 5-CH_3FH_4 was the major metabolite. The authors concluded that an important role of ascorbic acid in human metabolism is to prevent the oxidation of tetrahydrofolate and keep the folate pool in metabolically active form[79]. Thus, ascorbic acid improves the utilization of at least two haemopoetic agents, iron and folic acid.

The role of ascorbic acid in preventing infections may be related to its effects on leucocyte metabolism and phagocytic activity[80–82]. The particle-stimulated shunt as well as glycolytic activities and bactericidal effect were markedly lower in the leucocytes of scorbutic guineapigs[81,82]. It is also believed to play a role in collagen synthesis by participating in the hydroxylation of protocollagen proline and lysine[83,84]. Its effect on wound healing may be related to this action.

The beneficial effect of ascorbic acid in preventing common cold is controversial[85–88]. According to one report from Canada[88], 1 g ascorbic acid daily had no effect on the frequency of cold, but it reduced the severity of the symptom. During colds, there is a shift of ascorbic acid from labile stores like leucocytes into plasma and perhaps tissues, indicating higher requirement. Ascorbic acid administration may potentiate the action of salicylamides by interfering with their metabolism via sulphuration pathway[89]. According to a recent suggestion, the anti-inflammatory response of ascorbic acid may be related to the inhibition of prostaglandin synthesis[90]. Aspirin promotes ascorbic acid uptake by leucocytes[90].

Ascorbic acid has been found to interfere with the formation of nitrosoamine from nitrate in processed meat. This observation is of considerable importance from the point of view of environmental health and meat industry[91]. This vitamin has also been reported to interact with trace metals[92].

Though studies in animals and man claim beneficial effects of vitamin C in fluorosis, in a controlled study, administration of 2 g ascorbic acid daily to fluorotic subjects had no effect on urinary excretion of fluoride[93].

The functional significance of ascorbic acid sulphate is not clear. It has antiscorbutic activity in the fish but not in the guineapig[72]. An enzyme ascorbate sulphate sulphohydrolase has been identified in monkey tissues but is not enough for regenerating ascorbic acid in nutritionally significant amounts[94]. Ascorbate sulphate is a good sulphating agent in the presence of mild oxidisers. It was, therefore, proposed that ascorbate sulphate may be involved in biological sulphation reactions and that the anti-atherogenic effect of ascorbic acid may be partly due to the formation of cholesterol sulphate leading to an elevated cholesterol clearance. While the transfer of ^{35}S from AS-2 ^{35}S to cholesterol has been reported in rat[95], ascorbic acid or ascorbate sulphate administration was found to have no effect on cholesterol elimination[71]. Perhaps, rat is not a good species for such an experiment.

Assessment of nutritional status — The assessment of ascorbic acid status is generally done by measuring the concentration of the vitamin in body fluids, such as blood (plasma and leucocytes) and urine[26]. Serum and urine values often reflect the immediate dietary intake and hence are of limited value but have been used widely in nutrition surveys. Serum levels less than 0·2 mg/100 ml are regarded as deficient, 0·2–0·29 mg low, and more than 0·3 mg acceptable[26].

The concentration of ascorbic acid in leucocytes may be a better estimate of its concentration in other tissues. With a sufficiently higher intake, tissues can be completely saturated. Leucocyte concentrations of 20-50 $\mu g/10^8$ cells have been reported in well-nourished adults. However, lower values of 16-17 $\mu g/10^8$ cells even after saturation have been observed in Indian subjects[70].

The average 24-hour urinary excretion of ascorbic acid by well-nourished adults ranges between 8 and 25 mg. The validity of using casual urine samples has been claimed. At best, they may be useful in surveys, but not for individual assessment. Load tests involving administration of large amounts of ascorbic acid have been tried but are not very reliable.

Ascorbic acid is a powerful reducing agent. A quick functional test based on the reduction of intradermally injected or lingually applied dichlorophenolindophenol has been suggested but is not specific[96]. A functional test based on the abnormal excretion of tyrosine metabolites needs to be investigated. According to the study quoted earlier, functional impairment in tyrosine metabolism was observed when leucocyte concentration of the vitamin was below 6·0 $\mu g/10^8$ cells in children and 4 $\mu g/10^8$ cells in adults[77].

Requirement in man — The question of ascorbic acid requirement for man has become one of the most controversial issues in nutrition. The requirement of a nutrient, such as a vitamin can be assessed by determining the amount required to saturate the tissues or the amount required to maintain good health and prevent biochemical or functional deficiency. Different estimates can be obtained depending on the type of functional criterion used. From the available literature, Brin[69] has summarized that less than 10 mg per day are needed to prevent scurvy, 20-25 mg for daily utilization, 50-100 mg for preventing neonatal tyrosinemia, 4-32 to 300 mg/day for wound healing,

more than 1 g for preventing common cold, 35 mg for having a beneficial effect on glaucoma and 50-60 mg/day for maintaining tissue saturation. However, leucocyte saturation of Indian subjects could be maintained with only 20-25 mg ascorbic acid per day[97].

According to some workers, the daily requirement of vitamin C should be judged from the rate of production of ascorbic acid by mammals that synthesize this vitamin. This amount varies between 3 and 19g/kg per day[98]. There are two fallacies in such an argument. Most smaller mammals consume more food per kg body weight and have a faster rate of growth. Hence, their vitamin requirement per kg body weight would be higher. The fact that the ascorbic acid synthesizing capacity was lost during evolution in the case of man and some animals may suggest that their need is lesser. The high requirement reported for the rhesus monkey may be to compensate for the oxidative catabolism of ascorbic acid in this species.

According to the recommendations made by the Indian Council of Medical Research in 1968, the daily allowance for vitamin C is 50 mg for adults. No additional allowance is given for pregnancy, but in lactating women, the recommended allowance is 80 mg per day (Table 1). Adequate vitamin C status of pregnant and lactating women in India, despite inadequate intake and multiple parity, has been observed[99]. Ascorbic acid utilization during pregnancy is better. This indicates that the utilization of ascorbic acid in women during stress and deficiency is improved markedly[87].

It is possible that daily intake of very high doses of ascorbic acid may pose problems. Higher excretion of hydroxyproline indicating greater catabolism of collagen has been reported in subjects receiving large doses of ascorbic acid[100]. Excess oxalic acid in urine may predispose to the formation of renal calculi. Mutagenic effect of ascorbic acid in the presence of copper has been reported in human fibroblast cultures[101]. Another danger (often not realized) with the administration of high doses of a vitamin is the withdrawal effect, if the intake is not maintained. Clinical symptoms of deficiency may appear if the level is reduced, though later, tissues become adjusted to the altered metabolic state. According to Chatterjee *et al.*[102], administration of 2-4 g of ascorbic acid daily to man leads to rapid increase in blood DHA levels; this is associated with hyperglycemia. These workers also claimed that DHA concentration tends to be higher in the blood of diabetics. The intake of anti-diabetic drugs had no effect on DHA levels in diabetics. *In vitro* studies suggest the possible inhibition of 2,3-diketogulonic acid decarboxylase in diabetes mellitus. The hypoglycemic effect of DHA may be related to degranulation of β-cells of islet detected during histological studies on the pancreas of guineapigs fed high cereal diets and large doses of ascorbic acid[103].

THIAMIN

Thiamin is widely distributed in nature. Whole cereals, especially the pericarp, is a rich source of the vitamin; hence thiamin deficiency is common in populations which subsist on polished rice. Certain foods, such as raw fermented fish, fermented tea leaves (chewed continuously as stimulant) and betelnut contain anti-thiamin factors, including enzymes called thiaminases, which destroy thiamin and hence, thiamin deficiency is sometimes seen in people who are habituated to such food items[68,103]. Cooking destroys the enzyme thiaminase. Secondary deficiency of thiamin can also arise due to chronic diarrhoea, severe diuresis (as in diabetes mellitus and diabetes incipedus), thyrotoxicosis, and degenerative arteriosclerosis[68]. Thiamin deficiency is very common among alcoholics, partly due to inanition and partly due to higher requirement of the vitamin. While small amounts of alcohol have a thiamin sparing effect, imbibition of large amounts increases thiamin requirement, since acetaldehyde formed from alcohol has to be converted to acetoin, whose formation is thiamin-dependent[104].

The severest form of thiamin deficiency leads to beriberi. It is a complex disease and assumes a variety of forms, such as wet or acute cardiac beriberi, chronic dry atrophic beriberi or polyneutritis, acute fulminating beriberi or pernicious type, infantile beriberi, Wernicke's encephalopathy or Korsakoff's psychosis, commonly seen in alcoholics. Beriberi is still seen in the rice eating populations of South East Asian countries. Epidemics of wet beriberi used to be very common in coastal Andhra Pradesh until 25 years ago, but for some unknown reasons, the disease in its acute form has disappeared from India. Chronic cases are, however, encountered, especially among pregnant women.

The incidence of subclinical thiamin deficiency is high in South East Asian countries like Thailand and Malaysia[105,106]. In Japan, the incidence has declined since 1960[107]. Recent studies in our laboratory suggest that while the biochemical evidence of thiamin deficiency is very frequently seen among the pregnant women in Hyderabad, it is not common in the general adult population[108–110]. In the Western countries, subclinical thiamin deficiency has been reported among the elderly and among pregnant women[111,112]. Generally, fetus is protected against maternal vitamin deficiency. However, when the maternal thiamin status is poor, infants can be born with subclinical thiamin deficiency[110,113].

Some inborn errors of metabolism have been shown to respond to high doses of thiamin[1]. Temporary amelioration of subacute necrotizing encephalopathy (SNE), a fatal genetic disease in children, with high doses of thiamin has been claimed by Cooper and Pincus[114]. The brain lesions in SNE are similar to those seen in Wernicke's encephalopathy. The biochemical aspects of this disease will be discussed later.

Metabolism and biochemical functions — Thiamin is absorbed from the upper small intestine by a specialized saturable transport process. Oral doses, above 2·5 mg, appear to be largely unabsorbed and there seems to be no justification for their use in vitamin therapy[115]. Japanese workers have prepared numerous derivatives of thiamin. Many of these, such as thiamin allyl disulphide, thiamin propyl disulphide, O, S-diacetyl thiamin, and O, S-dibenzoyl thiamin are

believed to be absorbed more easily than thiamin hydrochloride. Thiamin propyl disulphide, a lipid-soluble allithiamin derivative, is very useful in the treatment of thiamin deficiency where rapid and sustained response is needed[116]. It can be reduced to thiamin non-enzymatically by SH groups containing substances, such as glutathione[117]. Enzymatic conversion has also been reported[118].

Thiamin and riboflavin, when present together, do not affect each other's absorption through the intestine, though both are converted to the phosphorylated forms during absorption[119]. Folate deficiency has been reported to affect thiamin absorption[120]. Malnourished alcoholics exhibit marked reduction in thiamin absorption. This would aggravate the state of thiamin deficiency. The impairment in thiamin absorption may be partly due to chronic malnutrition and partly to the effects of alcohol *per se*[121].

In the fetus, the highest concentrations of thiamin are found in the nervous tissue, but from the 5th month of fetal life, liver and heart begin to show higher concentrations and in postnatal life highest concentrations of thiamin are found in the liver, heart and kidney.

Cord blood generally contains higher concentration of thiamin and higher transketolase activity than mother's blood, suggesting that fetus is relatively protected against maternal malnutrition[110,113].

Thiamin is metabolized extensively in animals and man. When [2-^{14}C] pyrimidine-labelled thiamin was administered to an adult volunteer, ten metabolites were detected in the urine, and when [2-^{14}C] thiazole-labelled thiamin was administered, 18 metabolites were detected[122]. Of these numerous metabolites, two, namely 2-methyl-4-amino, 5-pyrimidinecarboxylic acid[122,123] and 4-methylthiazole-5-acetic acid[122–124] have been identified consistently. Yeast has been shown to synthesise thiamin molecule from the purine and pyrimidine metabolites of thiamin[125]. This has been developed into a method for detecting thiamin metabolites in the urine. At low dietary intakes, intact thiamin disappears from the urine and only metabolites are excreted[125].

Thiamin probably plays three major roles in mammalian systems. One is concerned with energy metabolism and involves α-keto acid decarboxylation. The second deals with synthetic mechanisms and involves transketolase activity. The third involves nerve conduction and is less well understood. Evidence has been accumulating to suggest that thiamin has a function in nerve conduction, which is independent of its coenzyme role[126]. Neuroactive reagents, such as acetyl choline, tetrodotoxin and ouabain, which cause a change in ion movements, produce a release of thiamin from spinal cord. These agents can also convert thiamin diphosphate (TDP) to thiamin monophosphate (TMP) and free thiamin in isolated nerve preparations. Thus, it has been poposed that cyclic dephosphorylation and rephosphorylation of thiamin compounds participate in mechanisms by which ions cross the nerve membrane. The presence of an enzyme phosphohydrolase which converts TDP to TMP has been described in membrane fragments. It is identical with nucleoside diphosphatase and is not activated by ATP[126]. Cooper and Pincus have suggested that the neurophysiologically active form of thiamin may be thiamin triphosphate (TTP) in contrast to the coenzyme form TPP[114]. Thiamin triphosphatase and thiamin pyrophosphate—adenosine triphosphate phosphoryl transferase have been isolated from the brain. The latter enzyme, earlier described in yeast, catalyzes the reaction TPP+ATP⇌ TTP+ADP[121].

In patients suffering from subacute necrotizing encephalopathy, a protein inhibiting the synthesis of TTP from TPP has been identified in the urine. In contrast to normal brains, those of autopsy patients are devoid of TTP[114].

The biochemical basis of the cardiac involvement or the neurological lesions of beriberi is not understood. The probable role of methyl glyoxal as a toxic factor proposed in the older literature has been refuted. The metabolism of methyl glyoxal is not impaired in thiamin deficient rats[127].

Assessment of nutrition status and requirements — Thiamin deficiency in man leads to fall in the thiamin content of blood and urine, and the transketolase activity of the erythrocytes[26,108]. Recently, the pyruvate decarboxylation rate of leucocytes was also reported to show a fall in thiamin deficiency[128].

The concentration of thiamin in blood is very low and difficult to estimate. According to Burch and coworkers, red blood cells do not lose their thiamin even when the intake of the vitamin is reduced to a point conducive to beriberi. However, Baker and Frank observed that thiamin concentration of whole blood is one of the most sensitive indices of thiamin nutriture[129]. These workers introduced a new microbiological assay, using the unicellular organism *Ochromonas danica*, for assaying thiamin in blood and reported a range of 25-75 ng/ml for whole blood and 15-42 ng/ml for serum[129]. At present, the reproducibility of this method in the hands of other workers is doubtful.

A rapid linear increase in urinary thiamin (measured in a 24 hour collection) occurs at intakes greater than 0·5 - 0·6 mg per day, or 0·3 mg/1000 cal. At lower intakes, the rate of increase is very slow[26,108]. Urinary excretion higher than 100 μg/day indicates adequate thiamin status[26]. Load tests which measure urinary excretion following 1 mg or 5 mg oral or parenteral load have also been developed[26,108]. Interpretation of urinary thiamin has to be done with caution, since it often represents the previous day's vitamin intake. Load tests, however, give a better estimate of the state of tissue saturation. Limited data on Indian subjects suggest that for a given intake of thiamin, urinary excretion in Indians tends to be higher than the values reported from other countries[108], and hence guidelines based on other studies may not be valid for Indians.

Since the biochemical functions of B-vitamins are known, functional tests can be applied for the assessment of B-vitamin status. The erythrocyte transketolase test (ETK) first described by Brin is used widely for thiamin. Apart from measuring the enzyme activity in the erythrocyte hemolysates, *in vitro* stimulation of the enzyme by the addition of coenzyme

TPP is also measured. This *in vitro* stimulation, referred to as the "TPP effect", is believed to be more specific for thiamin deficiency, since it represents the under-saturation of the apoenzyme transketolase for the coenzyme TPP. TPP effect more than 15% indicates thiamin deficiency[26].

In a controlled study on human volunteers in whom thiamin deficiency was induced by dietary means, it was observed that, in the earlier stages of thiamin deficiency, the TPP effect is marked, but as the deficiency progresses, the enzyme activity cannot be fully restored by the addition of TPP, suggesting actual or functional loss of the apoenzyme[108]. In the opinion of the author, the enzyme activity *per se* rather than the TPP effect is a more reliable indicator of thiamin deficiency in a population that may be suffering from chronic vitamin deficiency[108–110].

Experiments in animals have shown that ETK test is not affected by deficiencies of other nutrients, such as riboflavin, pyridoxine, folic acid, ascorbic acid and protein[130,131]. According to one report, ETK activity tends to be elevated in pateints suffering from vitamin B_{12} deficiency[26]. Diseases of the liver and gastrointestinal tract tend to lower the enzyme activity[26]. Despite these limitations, it can be said that ETK is a good index of thiamin nutritional status.

In vitro addition of TPP cannot stimulate liver transketolase activity even in thiamin deficient animals[131]. However, when thiamin is administered to thiamin deficient rats, a significant increase in the transketolase activity of the liver occurs within 3 hr, which cannot be blocked by the administration of the inhibitors of protein synthesis, such as actinomycin and puromycin, suggesting that in the livers of the deficient animals some apotransketolase occurs in an inactive form which cannot be activated by the *in vitro* addition of TPP to the liver homogenate, but which can be activated by the *in vivo* administration of thiamin[132]. Several days of thiamin feeding is necessary for the complete restoration of the transketolase activity of a thiamin deficient animal.

Since thiamin is mainly involved in the metabolism of carbohydrates, its daily requirement is expressed on the basis of calories. In a controlled study on human volunteers[108], ETK activity showed a linear rise with increasing intakes of thiamin up to 0·2 — 0·3 mg thiamin/1000 kcal. After that, the enzyme activity began to plateau. At this level of intake, urinary excretion of thiamin showed a sharp rise. Based on these two parameters it would appear that the minimum daily requirement for thiamin is between 0·2 and 0·3 mg/1000 kcal. This estimate agrees well with the earlier estimates based on diet surveys. After making allowances for cooking losses, Gopalan and Narasinga Rao suggested the daily allowance to be 0·5 mg/1000 kcal (Table 1). Additional allowance of 0·2 and 0·3 mg /day was recommended for pregnancy and lactation respectively (Table 1). Recent studies show that additional supplements may be required for women who use oral contraceptive steroids.

RIBOFLAVIN

The best food sources of riboflavin are milk, liver, meat, eggs and some of the green leafy vegetables. Cereals are a poor source of the vitamin. In Indian diets, pulses contribute a significant amount of riboflavin. Apart from dietary inadequacy, deficiency may arise from chronic diarrhoea, use of antimetabolites and antibiotics and in liver diseases from poor utilization of the vitamin.

The consequences of riboflavin deficiency in man are not as serious as those of some other vitamin deficiencies. The typical symptoms of ariboflavinosis are the triad of orolingual lesions—angular stomatitis (inflammation of the angles of the mouth), glossitis (inflammation of the tongue), and cheilosis (drying and ulceration of the lips). Seborheic dermatitis in the nasolabial folds and scrotal dermatitis are also seen in cases with severe deficiency. Corneal vascularization has been reported in riboflavin deficiency, but it is a subject of controversy.

Experimental deficiency of riboflavin as well as pyridoixine produces similar orolingual lesions. Recently, it was reported that these lesions (very commonly seen in Hyderabad area) respond to treatment with either riboflavin or pyridoxine or both the vitamins. The biochemical interrelationship between these two vitamins will be discussed later.

The prevalence of oral lesions of riboflavin deficiency is very high in India, particularly in the Southern States. In a survey of preschool children, the incidence of clinical riboflavin deficiency was found to rise from 1·6% at 1–2 years to 7·5% at 4–5 years, the average being 5·2% (ref. 133). In South India, 44% of pregnant women had clinical riboflavin deficiency[134]. The data collected by us in recent years have shown that almost all pregnant women and majority of non-pregnant women of low and middle income groups in Hyderabad have biochemical riboflavin deficiency. Vitamin B_2 deficiency during pregnancy has been observed even in European women[135].

Absorption, transport and utilization of riboflavin — Studies in animals have shown that riboflavin is absorbed from the proximate part of the small intestint by a specialized transport process. The transpore process is capacity limited[136]. FMN is dephosphorylated to free riboflavin in the lumen and rephosphorylated in the mucosa. It initially appears in the blood but is rapidly dephosphorylated to riboflavin. The gastrointestinal absorption of riboflavin, particularly when present in doses higher than 10 mg is better, in the presence of food[136]. This may be due to the stimulation of bile flow by food (since bile facilitates absorption of riboflavin) or increase in intestinal transit time. Thiamin and folic acid have no effect on riboflavin absorption, but a combination of vitamins C and D and calcium has been reported to diminish riboflavin absorption. This subject has been reviewed recently[136,137]. Several drugs and hormones modify riboflavin absorption and utilization. These effects will be discussed later.

Yagi[138] prepared a number of fatty acid esters of riboflavin which are expected to function through the liberation of riboflavin by the action of pancreatic lipase in the small intestine or non-specific esterases elsewhere. Derivatives, such as riboflavintetrabutyrate and riboflavintetranicotinate appear promising,

since they are able to sustain body levels of the vitamin in rats. Riboflavintetrabutyrate being a lipid-soluble derivative has deposit type action. Blood levels are maintained better and urinary excretion is slower when riboflavintetrabutyrate is compared with free riboflavin. The direct absorption of a major portion of riboflavintetrabutyrate has also been observed. The ester is deposited in the liver and slowly hydrated to riboflavin and butyric acid.

The transport, distribution and elimination of a vitamin are determined by the extent of its binding to plasma proteins. Thus, the genetic absence of a riboflavin carrier protein in a strain of Leghorn chickens leads to riboflavin deficiency and subsequent embryonic death[139].

Studies of flavin binding to human plasma have been done only with riboflavin and FMN. Both these flavins can interact with all the serum proteins of man. Albumin, however, accounts for most of the binding[136]. The interaction may involve hydrogen bonding between the 3-amino group of the isolloxazine ring of riboflavin and tyrosine hydroxyl groups of the protein. The association constant for FMN binding to human albumin is 20 times larger than that for riboflavin.

Renal excretion of riboflavin in man involves glomerular filtration, tubular secretion and tubular reabsorption. The amount excreted depends upon the amount present in the body. If a large dose is administered, nearly all of it is recovered in the urine. Degradation products of riboflavin have been detected in human and goat urine, but most of it is believed to be of bacterial origin[140]. Very recently, however, Christensen in Copenhagen and Tillotson and Karcz in USA have observed that a small but significant fraction of administered [2-^{14}C]-riboflavin yields ^{14}C-metabolites from the rat (McCormic, D.B., personal communication).

The ingested flavin is stored as coenzyme in association with enzyme proteins to function catalytically in biological oxidation-reduction reactions. Most tissues have the enzymes for the synthesis of FAD and FMN. Apart from FAD and FMN, a number of less common forms of flavins have been identified in tissues of riboflavin-dependent higher animals[141]. In most flavin enzymes, FMN and FAD are bound to the protein non-covalently. However, now at least eight flavin enzymes with covalently linked FAD are known[142].

Metabolism in cancer — This subject was reviewed recently[143]. Certain observations in man show that riboflavin deficiency induced by dietary means in combination with galactoflavin has an anti-tumour effect in man. The mechanism is not known. One possibility is starvation of the rapidly multiplying tumour of the flavin coenzymes that are vital for metabolism. Riboflavin deficiency in rats limits the growth of certain types of spontaneous and transplanted cancers, but enhances azo dye carcinogenesis. It has been shown that the neoplasm is resistant to riboflavin deficiency and maintains its FAD levels[143]. Highly malignant Novikoff hepatoma grown intraperitoneally does not modify hepatic riboflavin, FMN or FAD in either normal or riboflavin deficient rats. Urinary excretion of riboflavin tends to be lower in cancer patients.

Assessment of riboflavin status — Until recently, the assessment of riboflavin nutritional status was done by measuring urinary and blood levels of the vitamin. Urinary excretion greater than 120μg/24 hr and erythrocyte concentration grater than 15 μg/100 ml cells are regarded as acceptable, low risk values[26].

Since erythrocytes lack mitochondria, only one or two flavin enzymes are present in these cells. Of these, glutathione reductase is the most important. It has been reported that the erythrocyte glutathione reductase activity (EGR) is sensitive to riboflavin nutriture in man[144–146]. In riboflavin deficiency, the enzyme activity shows a fall, but the *in vitro* stimulation of the enzyme by the addition of FAD referred to as "FAD effect" or "activation coefficient" shows an increase. FAD effect higher than 25% (activation coefficient greater than 1·25) is regarded as indicative of riboflavin deficiency[26,144–146].

The EGR test reveals a very high incidence of riboflavin deficiency in population around Hyderabad[109]. In many subjects, the enzyme activity is as low as 30% of normal values. This test is more sensitive than erythrocyte riboflavin concentration, and more reliable than urinary riboflavin. Deficiency of other vitamins, such as thiamin, folic acid and vitamin C in animals had no effect on the EGR test[147]. Pyridoxine deficiency, however, produced a marked elevation in RBC riboflavin but a fall in EGR, activity and no change in FAD effect[147]. The fall in EGR activity in pyridoxine deficiency may be due to fall in apo-EGR.

Increase1 EGR activity with a high degree of saturation with FAD has been reported in the red cells of patients with severe uremia, cirrhosis of liver and glucose-6-phosphate dehydrogenase deficiency[148]. Higher activity has also been reported in iron deficiency anaemia[149].

According to some reports, the RBC riboflavin concentration of cord blood tends to be 4-5 times higher than that of maternal blood[113]. However, in a recent study on Indian women of low income groups and their new-born infants, the riboflavin concentration of the maternal and the fetal blood was found similar[110]. It is possible that, in malnutrition, the transport of riboflavin across the placenta is impaired. EGR activity was higher and the FAD effect lower in the fetal blood compared to the maternal blood. This would suggest that perhaps the cord blood EGR has higher affinity for the coenzyme.

Physiological implications of glutathione reductase defect in riboflavin deficiency — Since glutathione reductase is one of the enzymes concerned with the maintenance of reduced glutathione levels in tissues, the physiological implications of its deficiency due to ariboflavinosis has been studied. The life span of ^{51}Cr-labelled erythrocytes of riboflavin-deficient and supplemented rats was the same even after the administration of an oxidant drug like acetylphenylhydrazine. Similarly, in one patient with EGR deficiency, treatment with riboflavin had no effect on the life span of labelled erythrocytes[150]. The stability of erythrocyte

GSH *in vitro* in the presence of acetylphenylhydrazine was similar in riboflavin deficient and normal subjects[151]. These results suggest that the enzymatic lesion due to riboflavin deficiency is not physiologically critical for the cell and riboflavin deficiency does not predispose a person to drug sensitivity as glucose-6-phosphate dehydrogenase deficiency does.

Requirement — The EGR test has been used to determine the minimum requirement of riboflavin in man. In a controlled study in human volunteers, the enzyme activity increased linearly with increase in dietary intake of the vitamin, but there was a plateau at an intake of 0·5 mg/1000 kcal[146], suggesting that minimum requirement is around 0·5 mg/1000 kcal. However, in another study, using a similar criterion, the minimum requirement was 0·7 mg/1000 kcal[152].

PYRIDOXINE

The term vitamin B_6 includes a number of vitamers, such as pyridoxine, pyridoxal, pyridoxamine and their phosphorylated forms. Experimental pyridoxine deficiency in animals leads to a variety of symptoms. Specific deficiency signs due to pyridoxine deficiency in man are not established. The clinical features of experimental deficiency produced by the administration of deoxypyridoxine, an analogue of vitamin B_6 are dermatitis, cheilosis, glossitis and polyneuritis. Vitamin B_6 deficiency in infancy is believed to affect the central nervous system, with the appearance of hyperactivity, behavioural changes, convulsive seizures, and possible mental retardation. In addition, anaemia as well as dermatitis may occur. Effects of vitamin B_6 deficiency on central nervous system have been reviewed recently[153].

In older individuals, the central nervous system changes are less apparent, although there may be some peripheral neuritis. However, there may be skin changes, reduction in immunity, formation of oxalate stones, anaemia, dental caries, hepatic cirrhosis and changes in lymphatic activity[154]. Pyridoxine therapy has been found to be beneficial in recurrent urolithiasis[155].

Several genetic abnormalities of pyridoxine metabolism possibly occurring as a result of derangements in the apoenzymes, and requiring large doses of pyridoxine (pyridoxine dependency) for correction have been identified. Examples of such enzymes are decarboxylase (central nervous system disorders), aminolavulenate synthatase (anaemia), Kynureninase (familial xanthurenicaciduria-urticaria) and cystathionase (mental retardation)[1].

Subclinical and clinical vitamin B_6 deficiency is invariably seen in pregnant women. This is partly related to fetal demand, but it is mostly due to hormonal effects which will be discussed later. In India, vitamin B_6 deficiency was not regarded as a public health problem. However, recent studies in our laboratory have shown that besides pregnant women, subclinical deficiency is common even in nonpregnant women and men. In fact, it appears that vitamin B_6 deficiency is as common as riboflavin deficiency. Apart from subclinical hypovitaminosis, clinical deficiency also occurs. Thus, oral lesions such as angular stomatitis and glossitis were recently found to respond to treatment with either riboflavin or pyridoxine[156,157]. In many cases, response to riboflavin was partial and required pyridoxine for complete healing. The biochemical interrelationship between these two vitamins will be discussed later. The vitamin B_6 status of the fetus tends to be better than that of the mother[110].

Serum pyridoxal phosphate (PLP) concentration tends to be lower in pregnant women suffering from hyperemesis gravidarum (vomiting during pregnancy). Marked clinical improvement with very hign doses of pyridoxine has been claimed[158]. Conversion of pyridoxine to PLP has been reported to be impaired in pre-eclamptic human placenta[159] and it has been suggested that parenteral administration of PLP may help this condition. More work is required to establish this.

Absorption, metabolism and biochemical functions of vitamin B_6 *in man* — Some of the earlier work on these aspects has been reviewed[159,160]. Pyridoxine, pyridoxal and pyridoxamine are utilized with equal efficiency by animals. *In vitro* and *in vivo* studies in rats have suggested that pyridoxine is absorbed rapidly by passive diffusion from jejunum. Intestinal alkaline phosphatase can hydrolyze the phosphorylated forms rapidly, but it is not known if that is a pre-requisite for their absorption.

In contrast to intestinal transport, the uptake of vitamin B_6 by cells, such as erythrocytes and Enrlich ascitis tumour cells appears to be an active saturable process. Phosphorylated derivatives are taken up poorly, if at all. Anderson *et al.*[161] have shown that red cells can abstract PLP from a saline solution, but not from plasma. More than 30% of vitamin B_6 in plasma is in the form of PLP, and it occurs in combination with albumin[162,163]. On the other hand, pyridoxal is only partially bound to protein and pyridoxine occurs free[162]. Organ removal experiments in dogs suggest that liver is the major if not the only source of plasma PLP[163].

Most tissues can form PLP from the other forms of the vitamin. In mammalian systems, the major pathway for the formation of PLP is : pyridoxine→ pyridoxine phosphate⟶pyridoxal phosphate. Pyridoxal kinase can convert all the three forms to their phosphorylated derivatives. Pyridoxaminephosphate oxidase can oxidize both pyridoxamine phosphate and pyridoxine phosphate to PLP. However, pyridoxamine phosphate has a lower K_m than pyridoxine phosphate[164]. It is an FMN dependent enzyme and its activity falls in the liver of riboflavin-deficient rat and erythrocytes of riboflavin deficient man[165,166]. An alternative pathway for PLP formation (pyridoxine→ pyridoxal→pyridoxal phosphate) has been shown to operate in brain and muscle[167].

The phosphorylated derivatives are readily hydrolyzed in tissues by cellular alkaline phosphatase[168]. The major urinary metabolite of vitamin B_6 is 4-pyridoxic acid. 4-pyridoxic acid 5 -phosphate has also been detected in human urine[169]. The tissue content of PLP is probably regulated by the binding of this coenzyme with the protein moiety[172]. Group specific proteases for PLP-dependent apoenzymes have been described[171]. These regulate PLP levels in tissues.

PLP serves as a coenzyme for a vast array of enzyme systems involving amino acid metabolsim, viz. aminotransferases, decarboxylases, desulphydrases, transulphurases, deaminases, racemases, and desmolases. Functions related to the metabolism of carbohydrates and fats have also been observed. Recently, PLP has been shown to be a cofactor for the enzyme O-phosphorylethanolamine phospholyase which degrades O-phosphorylethanolamine (OPE) to ammonia and ethanol[172]. In vitamin B_6 deficiency, there is a fall in the activity of this enzyme in the rat liver and increased urinary excretion of OPE. This biochemical lesion may be related to some osteoporotic bone diseases in rat and man.

Interrelationship between riboflavin and pyridoxine — Since lesions of the mouth such as angular stomatitis and glossitis were found to respond to treatment with either riboflavin or pyridoxine[156,157], and since the formation of PLP in mammalian tissues is catalysed by the FMN enzyme, pyridoxamine phosphate oxidase, it was postulated that this oral pathology is due to cellular deficiency of PLP. Subsequent experiments to varify this hypothesis show, that, in riboflavin deficiency, the activity of pyridoxaminephosphate oxidase in rat liver and human erythrocytes exhibits marked reduction, thereby limiting the synthesis of PLP *in vitro* as well as *in vivo*[165,166]. Simultaneously, pyridoxal phosphatase activity also falls in riboflavin deficiency, and the PLP levels in tissues remain within the normal range. This level, however, is insufficient to meet the cellular requirement of PLP in riboflavin deficiency, because, by a mechanism not yet understood, the levels of some PLP enzymes such as transaminases and serine dehydratase go up, increasing the intracellular PLP-binding sites[173]. As a result, an unequal distribution of PLP between its enzyme systems occurs and pockets of functional deficiency appear. Thus, the activities of homoserine dehydratase and perhaps kynurininase fall. Treatment with high dose of pyridoxine is able to push up the PLP levels, producing clinical response. Treatment with riboflavin corrects the biochemical lesion in pyridoxine metabolism and clinical improvement is seen.

Assessment of vitamin B_6 *status and requirement* — Vitamin B_6 deficiency results in marked fall in urinary excretion of vitamin B_6 (pyridoxal, pyridoxine and pyridoxamine) as well as pyridoxic acid. In adults, urinary excretion of vitamin B_6, higher than 20 μg/g creatinine, is regarded as acceptable. Excretion in children is higher. Blood PLP levels also fall in deficiency. Since the advent of sensitive assays for the estimation of blood PLP (based on apotryptophanase or apotyrosine decarboxylase coupling with PLP), it is being felt that plasma PLP may prove to be a good index of pyridoxine status. This needs experimental verification.

Among the functional tests known, the two most commonly used tests are tryptophan load test and the activities of erythrocyte aminotransferases. PLP is required at several points in the tryptophan-niacin pathway. Of the various enzymes involved, kynaurinase, which converts 3-hydroxykynurenine to 3-hydroxyanthranilic acid, is most sensitive to pyridoxine deficiency. When a load of tryptophan (generally 2g) is administered, deficient subjects show higher urinary excretion of the metabolites such as kynurenic acid and xanthurenic acid, even though their formation is also catalysed by PLP-dependent transaminases.

The activities of aspartate aminotransferase and alanine aminotransferase in erythrocytes have been shown to be very sensitive to vitamin B_6 deficiency. Both the enzymes show *in vitro* stimulation with the coenzyme PLP and the extent of this stimulation is significantly raised in vitamin B_6-deficient subjects. Aspartate aminotransferase stimulation higher than 50% and alanine aminotransferase stimulation greater than 25% are regarded as deficiency. However, the cutoff points are not well established.

The enzymatic tests are now extensively used in nutrition surveys. However, their interpretation should be done with caution, since aspartate aminotransferase activity is found to increase in riboflavin deficiency[174] as well as in women using oral contraceptives[26,109], probably due to alteration in the level of apoenzyme aspartate aminotransferase.

In vitamin B_6 deficiency, methionine loading induces a marked elevation of cystathionine excretion, which can be prevented by pyridoxine supplementation. This test has received only limited trial[175,176], but is too complicated and expensive. Pregnant women in India were found to have higher cystathionine excretion[176]. Pregnant women also show a marked impairment in tryptophan metabolism, which can be corrected with very high doses of pyridoxine.

Pyridoxine requirement is related to the protein intake. Human requirement of the vitamin has been suggested as 1·4 - 2·5 mg per day[154]. Requirement is believed to increase with age and is modified by the use of drugs such as isoniazide and oral contraceptives. Pyridoxine requirement of patients suffering from Parkinsonism and treated with levodopa has become a controversial issue[177].

Interaction of Vitamins with Drugs and Hormones

Man is living in a constantly changing chemical environment. Use of various drugs, such as antibiotics, antitubercle agents, anti-inflammatory drugs, anticonvulsants, laxatives, sedative , antifertility agents, etc. contribute significantly to the changes in the chemical environment. Drugs have been found to interact with vitamins by affecting their absorption, metabolism and utilization and their use is sometimes associated with the development of vitamin deficiency diseases.

Numerous studies in animals show that while small doses of antibiotics have a sparing effect on B-vitamins, high doses of broad spectrum antibiotics such as tetracyclins produce B-vitamin deficiency by destroying the micro-organisms in the gut. Yagi *et al.* have reported that tetracyclins interfere with the conversion of riboflavin to its coenzymes[178]. Aureomycin, when administered to human volunteers, was found to increase the urinary excretion of thiamin, riboflavin and N-methyl nicotinamide, but produced a fall in the urinary excretion of thiamin metabolites and in erythrocyte transketolase activity[179]. These data suggest that aureomycin impairs vitamin utilization.

Prednisolone, an anti-inflammatory steroid has also been reported to affect riboflavin metabolism[180]. In humans, the use of thalidomide during pregnancy has been found to produce fetal malformations, resembling those seen in rats due to riboflavin deficiency[181]. The structural similarity of thalidomide to riboflavin has led to the suggestion that this drug acts by antagonising riboflavin[181].

Isonicotinic acid hydrazide (INH) used in the treatment of tuberculosis is a well-known pyridoxine antagonist. INH forms hydrazones with pyridoxal or pyridoxal phosphate whch are lost in the urine[182]. INH has also been shown to competitively inhibit pyridoxal phosphate-dependent enzyme[183]. Significant improvement in the pyridoxine status of patients treated with INH following treatment with 50-100 mg pyridoxine for 15-30 days was reported recently[184].

Anticonvulsant drugs have been reported to produce folic acid deficiency by interfering with folate absorption[185].

In the last few years, numerous reports and review articles have appeared on oral contraceptive (OC) steroids and vitamin interactions[109,186–188]. It has been shown that women using OC develop biochemical evidence of pyridoxine deficiency, as judged by impaired tryptophan metabolism. Many workers have confirmed this observation[109,188]. The abnormality in tryptophan metabolism can be corrected by the administration of large doses (10-100 mg) of pyridoxine daily[189–191].

The biochemical basis of this defect is not understood. *In vitro* and *in vivo* studies suggest that estrogen or its metabolites interfere with the binding of PLP with kynurenine aminotransferase[186]. Similar inhibition of kynureninase may occur in *in vivo*. OC-treated women and female rats are reported to have elevated levels of transaminases, such as aspartate aminotransferase in their tssues[109,186]. This can raise the reqirement for vitamin B_6.

Some workers feel that since among numerous vitamin B_6-dependent functions, OC selectivity alters tryptophan metabolism, it may not be necessary to give pyridoxine supplements to women using OC[192]. However, recently, vitamin B_6 supplements have been reported to improve the carbohydrate tolerance of women using OC[193].

Oral contraceptive steriods have also been shown to decrease the blood levels of folic acid, vitamin B_{12}, ascorbic acid and riboflavin and decrease the urinary excretion of riboflavin in women[109,186,188194,]. Enzymatic evidence of altered thiamin and riboflavin status has been reported[109,197] Here again, the biochemical mechanisms responsible for the observed changes are not understood. Recent studies in female rats suggest that OC elevate the riboflavin requirement by acting at the level of flavoprotein enzymes[194]. The activities of some flavin enzymes, such as D-amino acid oxidase and glutathione reductase tend to be elevated in the livers of OC-treated female rats, whereas activities of other flavin enzymes, such as NADH-dehydrogenase tend to fall.

Diminished folate absorption, and increased folate clearance are the two possibilities advanced for the observed fall in blood folate levels of women using OC[186,195].

In view of the marginal or poor vitamin status of the women in India, it may be necessary to fortify pills with vitamins. Based on clinical and biochemical studies, we have recently recommended additional supplements of 2-3 mg thiamin and riboflavin and 10 mg pyridoxine to women using OC[189]. These supplements can mitigate the contraceptive effect.

Unlike most other vitamins, plasma levels of retinol increase in women using OC[109,188]. This increase is believed to be due to the rise in retinol binding protein and the transport of the vitamin from the liver into circulation[196].

In view of the fact that OC are used by healthy young women for prolonged periods, their interaction with nutritional and other health variables acquires special significance.

Important interactions among vitamin and other hormones have also been described. Thus, riboflavin deficiency in animals has been shown to cause adrenal cortex stimulation initially followed by exhaustion and atrophy[197]. Thyroxine has been reported to enhance the synthesis of flavin coenzymes from riboflavin[198].

Summary

Vitamin deficiency diseases, such as xerophthalmia (leading to blindness), rickets, beriberi, pellagra, and oral lesions, continue to contribute significantly towards moibidity among the poor in develop]ng countries. The incidence of beriberi and scurvy has come down in India in recent years. Blindness due to vitamin A deficiency may be prevented through administration of prophylactic doses of this vitamin to children of ages between 1 and 5 years. Vast gaps exist in our understanding of the biochemical and molecular functions of fat-soluble vitamins and vitamin C. A correlation between the clinical features of vitamin deficiencies and known biochemical functions is yet to be established. Important discoveries in the area of vitamin A transport, active metabolites of vitamin D, molecular mode of action of vitamin K and metabolism of vitamin C have been made in the last decade. Metabolic interactions between vitamins and some commonly used drugs, such as antimetabolites, antibiotics and oral contraceptives can raise the vitamin requiement and lead to vitamin deficiency in man. For every frank case of vitamin deficiency there are many others who suffer from subclinical deficiency. Functional tests based on enzymatic functions of vitamins have been developed to assess the nutritional status of some B-complex vitamins. These help to identify subclinical deficiency in man.

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REVIEWS

CHEMICAL THERMODYNAMICS—4, edited by J. Rouquerol and R. Sabbah (Centre de Recherches de Microcalorimetric et de Thermochemie du C.N.R.S., Marseille, France; Pergamon Press Ltd, Oxford), 1976. Pp. 97 (245-331); Price $ 16·00; £ 8·90

This is a compilation of the seven plenary lectures presented at the Fourth International Conference on Chemical Thermodynamics held in Montepellier, France during 26-30 August 1975. These focus attention on the recent developments in techniques and analysis in experimental thermodynamics. Minicalorimeters of capacity 0·3 cm^3 and error $\pm 0 \cdot 2 \sim 0 \cdot 3\%$ in the range 12-300°K have been fabricated.

The first chapter by C.E. Vanderjee deals with recent problems and strategies in thermochemistry. The title is too general, although the author has mainly confined to areas of his interest. The interest seems to be developing in thermochemical studies of slow processes and in building small calorimeters. Attention has been drawn to current problems such as those relating to (1) standard state of solids, (2) measurement of thermal response with desired sensitivity and accuracy, and (3) determination of the amounts of substance reacting and the identification of the actual processes which occur during the calorimetric experiment.

The second chapter on heat capacities in critical regions is due to F. Grønvold. It presents a good compilation of heat capacity results in the liquid gas and the magnetically critical regions. Reference has also been made to ac calorimetry.

P.J. Spencer discusses high temperature metallurgical thermodynamic data. Although it is not exhaustive, still it presents an interesting account of high temperature calorimeters, including pulse calorimeter. Precipitation calorimetry and E.M.F. measurements have been touched. briefly. An interesting discussion on application to metallurgical processes has also been included.

G.M. Schneider's review on thermodynamics properties of fluid mixtures of non-electrolyte is timely, since there is very little activity in this field.

Some aspects of thermodynamic studies on biological macromolecules have been discussed by Privalov in the fifth chapter. These relate to the application of scanning and reactive calorimetry methods. This would stimulate greater thermodynamic activity in the area of study of biological molecules.

There is a good critical review of the effects of molecular size and shape in solution thermodynamics by D. Patterson in the sixth chapter which would be of considerable interest to persons engaged in research on solution thermodynamics.

The seventh chapter is on surface thermodynamics in France by J. Rouquerol. The reviewer feels that it should have been in English so that the larger section of readers coula benefit from it.

The last chapter by Takahashi again deals with heat capacity measurements. It describes some of the latest techniques, such as laser-flash calorimetry and the high-resolution calorimetry developed in Japan.

The articles written by different experts are authoritative and provide lot of informative material regarding recent developments in thermochemical and thermodynamic measurements. The book would be useful to research workers in the area of experimental thermodynamics in academic institutions as well as in the industry.

R.P. RASTOGI

THE ANALYTICAL CHEMISTRY OF SYNTHETIC DYES, edited by K. Venkataraman (John Wiley & Sons Inc., New York), 1977. Pp. xvi + 591

This book combines in one volume the various analytical methods that have been employed in the study of dyes and their intermediates. The volume is very ambitious in its scope and includes very sophisticated methods such as high pressure liquid chromatography and X-ray powder diffraction.

The volume begins with an introductory chapter by the editor followed by (a) chapters on separation methods such as thin layer chromatography, paper chromatography and electrophoresis, high pressure liquid chromatography, gas chromatography, and (b) chapters on structural analysis methods such as infrared spectroscopy, NMR spectroscopy, mass spectroscopy, X-ray powder diffraction and chemical degradation. In between is a chapter on solution coloristics. The subsequent three chapters deal with the actual use of the different methods in isolation of dyes from commercial samples and determination of their structures. To specialists in this subject, these three chapters will be of immense value, since they help to consolidate the information given in previous chapters. Finally, there are chapters on (i) identification of dyes on textile fibres, (ii) identification of organic pigments on substrates other than textile fibres, (iii) analysis of food, drug and cosmetic colours, (iv) analysis of hair dyes, (v) quantitative analysis, and (vi) analytical techniques for ecological and toxicological monitoring.

Prof. Venkataraman has done a wonderful job of getting together so many experts in the field to contribute these chapters. The material has been well organised and carefully edited. Any criticism of such a book is, therefore, made with some hesitation. I felt that such a book being a unique reference volume should have provided more information in tabular form rather than referring the reader to the original papers. A notable exception is the chapter on NMR spectroscopy which gives considerable amount of information in that area with illustrative examples and tables. There seems to have been considerable amount of condensation due to limitations of space. This could have been avoided by either limiting the scope of the volume or by dividing it into two parts.

There is no separate chapter on UV visible absorption spectra of dyes, though the chapter on solution coloristics makes some reference to this subject. It would have been very useful to have a separate chapter on UV visible spectroscopy with useful tables on absorption data of various classes of synthetic dyes. Similarly, the chapter on quantitative analysis refers throughout to spectrometric determination but little information is given on chemical methods. There could have been a separate section in this chapter dealing with chemical methods and reference to recent innovations in this area.

On the other hand, one may well question the value of chapters on high pressure liquid chromatography and X-ray powder diffraction, as they are not likely to be used with any degree of frequency by most dyestuff chemists.

The chapter on mass spectroscopy is particularly disappointing, since there are no actual examples of major fragmentation pathways illustrated for different structural types. The chapters on separational methods on the other hand present the relevant material in fairly good detail.

There is little doubt that this volume will find a large number of interested readers and that it will occupy a special place in libraries devoted to applied chemistry.

S. SESHADRI

FOOD MICROBIOLOGY : PUBLIC HEALTH AND SPOILAGE ASPECTS, edited by Mario Defigueiredo and Don Splittstoesser. (The AVI Publishing Co. Inc., Westport, Connecticut), 1976. Pp. ix + 492

The objective of the book, as the authors have claimed, is to impart information relating to microorganisms that are important in foods either because of the illness they cause or the spoilage of foods they bring about. One notable feature of the book is its treatment of the subject. Unlike other textbooks the chapters are oriented towards individual species or groups of microorganisms rather than subjects like preservation methods or types of food.

The book is divided into sixteen chapters, each chapter being contributed by an expert in the field. The different chapters are: Statistics in microbiological quality control, *Staphylococcus aureus, salmanella* and *shigella, Clostridium botulinum, Clostridium perfringens, vibrioparahemolytiens,* Toxigenic fungi, Viruses, Coliforms, enterococci and other microbial indicators, Gram positive non-spore-forming rods, Gram negative non-spore-forming rods, Aerobic and Anaerobic spore-forming bacteria and food spoilage, Micrococci, Lactic acid bacteria, Yeasts, and Moulds.

Each chapter is excellently written, keeping in view the organisms habitat bases for their recognition and the factors that contribute towards their growth or death in foods.

The first chapter on statistical microbiological qnality control is written to familiarize the microbiologist with the applications of statistics in the microbiological quality control of foods.

The chapter on *Staphylococcus aureus* deals with this microorganism exhaustively; nearly 25% of the books deals with this important aspect of food borne disease-staphyloenterotoxicosis. The chapter is very well supported by a well documented bibliography comprising 638 references. The other chapters are also written equally well by the experts in the field.

This book is ideal for the purpose of educating food industry personnel who have the responsibility of safeguarding their industry's products against public health hazards and spoilage. It is equally useful to students of food microbiology who would like to get specialized in public health microbiology. The only drawback of the book is that separate chapters have not been devoted on *Bacillus cereus,* and enteropathogenic *Escherichia coli.* Interest in these organisms is catching up fast.

On the whole, the book makes interesting reading for all those concerned with food microbiology, in general, and public health microbiology in particular.

C.T. DWARAKANATH

FOOD COLLOIDS, edited by Horace D. Graham (The AVI Publishing Co. Inc., Westport), 1977. Pp. viii+ 426. Price $31·00

The volume under review attempts to present an overview of the hydrocolloids of plant, animal and microbial origin which are extensively used in food processing industry. The general principles of colloidal behaviour as applied to food additives have been covered in the introductory chapter. This gives a basis for understanding the rationale of the use of hydrocolloids in food industry. The hydration theory is very elegantly explained. Physical methods used in quantitating colloidal behaviour have been discussed. This is followed by 13 monographs on milk proteins, concentrated seed proteins, egg proteins, meat proteins, fish proteins sulphated seaweed polyacctarides, cellulose hydrocolloids, pectic substances, algin, modified starches, xanthan gum and plant gums. These monographs represent uptodate reviews of the current knowledge on the biochemistry of these substances as relevant to the food industry. The analytical methods currently used for hydrocolloids have been described in great detail. The book will prove to be a very useful reference work for those interested in the colloidal aspects of food processing.

C.R. KRISHNA MURTI

FOOD PROTEINS, edited by John R. Whitaker and Steven R. Tannenbaum (The AVI Publishing Co. Inc., Westport, Conn., USA), 1977. Pp. xii+602. Price $36·00

Here is yet another book on food from the now prolific AVI Publishing Co. Starting two decades ago by publishing occasional symposia proceedings at very low prices as a service to food scientists, AVI now seems to rival the professionals in output and price. Indeed the outpouring of books by competing publishers on the same subjects, sometimes with chapters by the same authors, is posing a real problem even to specialized technical libraries. It is difficult to choose between them, and there are hardly ever the resources to buy them all. And this volume, we are informed, is to be the first of a series of basic

symposia in food science and technology. Food proteins have been chosen, like Abou Ben Adhem, to lead all the rest.

There are 23 articles in all. One group covers basic aspects of specific proteins (muscle, milk, egg, cereals, legumes and single-cell protein) and another is concerned with protein structure, degradation, separation and analysis. A further lot covers protein quality and evaluation, functional properties and nutrition, and yet another protein technology and texturization. Finally there is a group of articles on production, future outlook, alternative sources of protein and anticipatory research needs. This is pretty wide spectrum for a single book, since each one aspect could, and does, support single monographs.

The reviews are mostly exceptionally readable, printed in large clear type with plenty of drawings and graphs and good explanations of basic concepts for the non-specialist reader. A few chapters may be specially mentioned. Hegsted once again makes out a case for his slope-assay ratio technique of measuring protein quality using three or more levels of protein ingestion, clearly an expensive procedure. Harper makes a trenchant attack on recent suggestions, particularly by Scrimshaw, that protein requirements have been underestimated by various national and international committees. A chapter by Kohler and Lyon on plant protein sources, which the authors admit is "limited", surprisingly totally omits mention of pulse proteins. There is a long final chapter on global research needs by Milner, Scrimshaw and Wang which, perhaps inevitably, takes off from an American standpoint; this catalogues exhaustively research needs in nutrition, toxicology, technology and agriculture, listing specific problems relating to various protein sources, followed by actual items of research for specific resource areas.

Food scientists and technologists in developing countries are likely to benefit most from the earlier review chapters rather than from those with a technological or futurological slant. Even in a competitive field the book can be recommended for institutional purchase.

K.T. ACHAYA

SPECIALITY : STEELS: RECENT DEVELOPMENTS: Chemical Technology Review No. 83, by G.B. Rothenberg (Noyes Data Corporation, Park Ridge, USA), Pp. xii + 270. Price $39·00

In the history of civilization, although the Iron Age dawned much later than the Copper and Bronze Ages, yet the production and utility of iron in its myriad forms—cast iron, mild and alloy steels, wrought iron—far outstrips any other metal whether in quantity of production or versatility of applications. Of iron Rudyard Kipling wrote :

> Gold for the mistress, Silver for the maid,
> Copper for the craftsman
> But iron, cold iron, is the master of them all.

Sir Robert Hadfield, the famous British metallurgist quoting an old proverb says, 'He who owns the iron of the world, will rule the world'.

Elaborating upon the inroads that steel (composed principally of iron) has made in our life, the author goes on to catalogue its applications: construction of buildings, bridges, railroads, aircraft, ships, automobiles, tools, cutlery, machinery, furniture, household appliances, spacecraft and material needed for national defence. This list is by no means complete. In the present volume, the author has presented an annotated bibliography of US patents relating to speciality steels. Included therein are the composition, and physical properties of steels and their manufacturing techniques. The subject matter has been divided into the following sub-heads: (1) Carbon steels, (2) High strength low alloy steels and other low alloy steels, (3) Tool steels, (4) Stainless steels, (5) Heat resistant steels, (6) Low carbon constructional alloy steels, (7) Silicon steel, electrical sheets, and (8) General steel making processes. Presentation of literature which is scattered in the vast US patent literature is a great service indeed to those planning to utilize the results already achieved or a researcher contemplating further research in this field.

The book is excellently indexed, printed and bound.

M.R. VERMA

Notes & News

Fission fragment detection by thin film capacitors

A new type of solid dielectric detector for fission fragments is reported from Laboratorio Dosimetria e Biofisica delle Radiazioni, Casaccia-CNEN, Rome, and Technion-Israel Institute of Technology, Haifa. The new detector is based on the counting of breakdowns in thin films of solid dielectrics.

The detecting element is a thin-film capacitor with a few hundred angstroms thick dielectric film and at least one electrode with thickness less than ~1000Å. It can be a metal-insulator-silicon sandwich or a metal-insulator-metal structure deposited on a glassy or ceramic substrate. In such a capacitor, each breakdown vaporizes a hole of sub-micron size in the insulator and a much larger hole in the thin electrode, thus avoiding a short-circuit. Investigations were carried out on metal-silicon dioxide-silicon structure (MOS).

Breakdown can be counted directly by a scaler, and visualization of the breakdown spots on the thin electrode can be carried out by a reflecting optical microscope. When the sample is observed under a microscope with a darkened field, sparks are seen on breakdown; these sparks are stronger, the thicker and larger the sample.

The property that makes thin-film capacitors suitable for detection is that fission fragments induce breakdowns at fields distinctly lower than the fields of the breakdown characteristics.

When compared with the damage-track detectors, the thin-film breakdown counters provide fast time response, avoid the need for chemical etching, but do not register the events, unless the fission fragments cross the insulating film when stressed with appropriate electrical fields.

This breakdown detector has finite applicability owing to gradual destruction by counting events. Therefore, an alternative use of this counter has also been explored for the detection of fissiou fragments by the measurement of current pulses at electric fields which are high, but still below the breakdown range for fragments. With capacitor areas of 2×10^{-2} cm^2, the pulses were insignificant when the oxide was relatively thin, but with 3800 Å thick oxide, fission fragments produced detectable pulses of about 10^{-15} C. These detectors offer the following advantages over the existing detecting systems: (1) they can be obtained with thicknesses appreciably lower than those of the semiconductors detectors and can withstand much larger applied electric fields; these characteristics are useful for timing and dE/dx measurements of highly ionizing particles; and (2) the associated signal processing equipment is simpler than for scintillation detectors.

The main problem in the use of thin insulator current-pulse counters appears to be the achievement of satisfactory signal-to-noise ratios [*Nucl. Track Det.*, **1** (1977), 63, 71].

Polyneutron systems

Of late, need has been felt for studying the possibility whether stable groups of neutrons — the polyneutron systems — can exist. As theoretical calculations, especially for higher number of nuetrons, are rather involved, the verification can be made experimentally.

A polyneutron system can most easily be detected by allowing it by studying its reaction with a chosen nucleus and studying the radioisotope formed thereby. This method was studied by Claude Detraz [*Phys. Lett.*, **66B** (1977), 333]. In this study, a tungsten target was bombarded with 24 GeV protons from the CERN synchrotron and an attempt was made to identify radioactive isotopes of Zn in a nearby block of that material. A sheet of Al shielded the Zn from any charged fragments,so that only neutral fragments could reach it. In this experiment ^{72}Zn was observed and so it was claimed that this provided a tentative evidence for the existence of bound neutral nuclei.

Experiments conducted to confirm this result have been carried out recently at the Los Alamos Meson Physics facility (LAMPF) [*Phys. Rev. Lett.*, **38** (1977), 1129]. In this set-up, a uranium target was bombarded with 800 MeV protons and a radochemical method was used to detect the presence of any bound polyneutron system that might have been formed. ^{208}Pb was used as a detector as its conversion to ^{212}Pb is an indication of the capture of a polyneutron system.

Their results, however, gave no evidence for polyneutrons; also they could work out upper limits to their production cross-section that are several orders of magnitude less than those found by Detraz. One possible reason for the nonformation of polyneutron system is the low energy tried at LAMPF. Another possibility is that Detraz might have observed bound systems of four neutrons, which could not have been detected in the Los Alamos experiment.

Silent DNA found within animal gene

Recent studies have shown that certain DNAs contain an intervening sequence of nucleotide bases that is not subsequently expressed in either the RNA or the protein made from them. It is believed that nature may have chosen to build this complex (animal) genome as a mechanism for creating a variety of genes from linear sequences.

Mouse β-globin genes were cloned and surprisingly it was found that β-globin DNA carries a large stretch of 'silent' DNA couched within it. This 'intervening sequence' runs for about 550 nucleotide bases and interrupts the β-globin between the sites that code for amino acids 104 and 105. Another, but smaller, intervening sequence probably exists in the β-globin gene; thus β-globin is divided into three discrete sequences.

The intervening sequence appears neither in the β-globin protein nor in its messenger RNA (the intermediate made from DNA and used by the cell to make the protein). It is speculated that the cell employs 'a bright and splice mechanism' to remove such inter-

vening sequences before they are turned into protein.

Though their purpose is not known, it is hoped that such intervening sequences offer great potential for variations within genes. For example, immunoglobulins might come from just such a variable mechanism within the genes. Each immunoglobulin contains a constant and a variable region of amino acids. Preliminary evidence is there that these two regions are separated by an intervening DNA sequence. That sequence might be involved in designating the shift from one type of immunoglobulin to another [*Chem. Engng News*, **55** (45) (1977), 6].

Computers and Chemical Engineering

This is the first issue of a new quarterly journal started by Pergamon Press Ltd from 1977. The journal aims to cover new computer methods or new applications in chemical engineering. Major areas of chemical engineering study that will be represented in the journal in the form of full length papers include; (1) process synthesis, analysis, and design; (2) dynamic analysis and control of chemical processes; (3) design methods for chemical engineering equipment, including chemical reactors, distillation columns, extractors, etc.; and (4) applications of computing and numerical analysis.

The contributions will be covered under four formats: (1) full length articles which will begin with three sections for the general reader: an abstract, a statement of scope, and a summary of conclusions and significance; (2) journal reviews; (3) short notes that will receive minimal review for quick publication; and (4) algorithms and programmes for general use by chemical enginners. Item (4) will be introduced in future issues. In this feature are covered the descriptions of chemical engineering programmes and subroutines, and where they are short enough, complete documentation and listings. For longer programmes, punched-card source decks will be available with documentation from AIChE headquarters in New York city at a price quoted in the programme announcement based on reproduction costs. The programmes submitted must be general-purpose. Authors will be given the opportunity to revise their programme(s) and documentation when appropriate.

The annual subscription for multiple-reader institutions is $ 64·00 (including postage and insurance); individuals whose institutions take out a library subscription may make a second or additional subscription at a reduced rate of $ 30·00.

Central Drug Research Institute, Lucknow

The report of the institute for 1975 records its R&D activities in the eleven major areas : antifertility agents, parasitic infections, amoebiasis, cholera immunology, viral infections, natural products, cardiovascular and nervous system disorders, disorders of carbohydrate and lipid metabolism, cancer, fermentation technology, and process development of drugs and pharmaceuticals.

An economic process for isolation of the anti-Parkinsonism drug L-Dopa from the beans of *Mucuna prurita* was standardized and demonstrated to the sponsoring firm. Dimethylbenzimidazole, an intermediate required for vitamin B_{12} manufacture, was under production by a small scale unit based on the CDRI process. This intermediate is an import substitution. Processes for paracetamol and lidocaine were released for commercialization.

A mutant strain of *Bacillus polymyxa* which is capable of giving a commercially viable yield of polymyxin B, an important antibiotic, has been developed.

A contraceptive cream containing total saponins of *Sapindus mukorossi* has been found to be effective for intravaginal use. It was found to be safe in toxicity studies carried out on rabbits. Centperazine, a new antifilarial compound under development, has been found to be without side-effects in single-dose phase I clinical trial and well tolerated up to 600 mg dose. A newly synthesized analogue of diethylcarbamazine showed promising antifilarial activity in animal tests. Limited fields trials with Centchroman, the new oral contraceptive were continued by the Union Ministry of Health and Family Planning in once-a-week dose as a possible prophylactic and single-dose *post-coitum*. The latter schedule showed encouraging results and the trials were being continued.

A potent β-adrenergic blocking compound has been synthesized which is marginally more cardioselective than propranolol with considerably less cardiac depressant activity. It also has antiarrhythmic activity. Clinical trial (phase I) of a new hypo-glycemic centpiperalone and phase II trial of the anti-inflammatory agent curcumin from *Curcuma longa* (*haldi*) were continuing satisfactorily. In teratogenic studies these compounds did not show abnormalities in mice and rabbits.

A heterocyclic quinone has shown activity against *Candida albicans* infection in mice at 0·625 mg/kg in oral administration.

A new animal model of filarial infection has been standardized by infecting the multimammate rat, *Mastomys natalensis*, with *Litomosoides carinii*, the cotton rat filarial parasite. This model will be useful for immunological and chemotherapeutic studies of filariasis.

Using various excystment agents in the presence of metabolic inhibitors, it has been observed that excystment of cysts of soil amoebae does not involve synthesis or transcript of DNA; *de novo* protein synthesis, presumably coded by pre-existing stable messenger RNA, appeared to be essential.

One hundred and three papers were published and twelve patents filed.

Announcements

● *A Course on Design, Analysis and Simulation of Chemical Processes* is being organized by the Department of Chemical Engineering, University of Roorkee, Roorkee during 11 June - 1 July 1978. The purpose of the course is to introduce chemical engineers to the fundamental aspects of mathematical modelling and the use of

these models for the design and optimization of chemical processes. Registration for the course is limited to 20 persons. Additional information can be had from Dr S.K. Saraf, Professor of Chemical Engineering, University of Roorkee, Roorkee 247 672.

● *An International Symposium on Marine Algae of the Indian Ocean Region*, sponsored by the Central Salt and Marine Chemicals Research Institute, CSIR, Bhavnagar, Dept of Science & Technology, Govt. of India, and the United Nations Educational, Scientific & Cultural Organization (UNESCO) will be held at Bhavnagar during the second week of January 1979. The major areas of discussion during the symposium will be (i) Survey, ecology, taxonomy, distribution, biology, physiology, and cultivation of marine algae; (ii) Chemistry of marine algae; (iii) Pharmaceuticals and energy from seaweeds; (iv) Biochemistry, nutrition and fertilizer aspects of marine algae; and (v) Utilization of marine algae for improvement of coastal areas.

The discussions will be followed by invited talks on marine energy farms, marine algal genetics, liquid seaweed fertilizer and pharmaceuticals from seaweeds.

An exhibition with the theme 'Marine algae in service of man' will also be held during this period.

Further information regarding the symposium can be had from Dr P.S. Rao, Secretary/Convener International Symposium on Marine Algae of the Indian Ocean Region, Central Salt and Marine Chemicals Research Institute, Bhavnagar 364 002.

● *The Thirteenth International Botanical Congress* will be held in Sydney in 1981. The programme of the congress will consist of 12 sections devoted to molecular, metabolic, cellular and structural, developmental, environmental, community, genetic, systematic and evolutionary, fungal, aquatic, historical and applied botany. There will be plenary sessions, symposia and sessions for submitted contributions (papers and posters). Field trips will include visits to arid and semi-arid regions, eucalypt forest, rain forest, heath, coastal vegetation (e.g. Great Barrier Reef, mangroves), etc., and specialist trips. Further details regarding the congress can be had from the Executive Secretary, Dr W.J. Cram, 13th I.B.C., University of Sydney, N.S.W. 2006, Australia.

● *A National Conference on Quality and Reliability* will be held at the Indian Institute of Technology, Bombay during the last week of November 1978. Further details regarding the conference can be had from the Organizing Secretary, Dr M. N. Gopalan, Department of Mathematics, Indian Institute of Technology, Powai, Bombay 400 076.

● *The Twentieth International Conference on Coordination Chemistry* (XX ICCC), 1979 sponsored by the Indian Chemical Society, Calcutta, and the Indian National Science Academy, New Delhi, will be held in Calcutta during 10-14 December 1979.

Further information regarding the conference can be had from The Secretary, Indian Chemical Society, 92 Acharya Prafulla Chandra Road, Calcutta 700 009.

● *A Workshop on Electrochemistry* organized by the Society for the Advancement of Electrochemical Science and Technology and the Central Electrochemical Research Institute, Karaikudi, will be held at Karaikudi during 19-30 June 1978. Lectures covering various sub-disciplines of electrochemistry will be organized during the workshop in which emphasis will be on the introduction of the subject from the elementary level to the recent trends in the field.

Further information can be had from Dr K.C. Narasimham, Secretary, Society for Advancement of Electrochemical Science & Technology, Karaikudi 623 006.

● *Toshiba Anand Fellowship for Research in Standardization*—The Lal C. Verman Research and Education Trust has instituted a fellowship for research work in the discipline of standardization at a recognized university or institution in India. The value of the fellowship is Rs 6000 which will be spread over a period of one year and paid in monthly instalments of Rs 500. Applications in prescribed proforma should be submitted to the Member-Secretary of the Trust, C/o Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110 002.

● *Dunlop Award for Fundamental Research in Rubber Technology*—M/s Dunlop India Ltd have instituted and annual award of Rs 10,000 for the best fundamental research work in rubber technology published in a particular year from April to March. The last date for the receipt of proposals for the current year's award is 30 June 1978. The same may be sent to the Director, Indian Rubber Manufacturer Research Association, Plot No. B-88, Road U, Wagle Industrial Estate, Thana 400 604.

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